ENHANCING THE EFFECTIVENESS OF THE *THAUMATOTIBIA LEUCOTRETA* (LEPIDOPTERA: TORTRICIDAE) STERILE INSECT TECHNIQUE RELEASE PROGRAMME

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Partial Fulfilment of the Requirements for the degree of

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of

RHODES UNIVERSITY

By

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September 2024

DECLARATION AND COPYRIGHT

I, **Michael Githae**, declare that this thesis is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature: 

DEDICATION

To my parents, Peter and Lydiah.
You have been my source of comfort and support during this unchartered journey and throughout my life.
Thank you for your love and caring nature.

I immensely love you.

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ABSTRACT

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is a key phytosanitary pest in the South African citrus industry. Due to its cryptic nature and its endemic presence in sub-Saharan Africa with a wide array of host plants, and eradication is not possible. However, the sterile insect technique (SIT) has been incorporated into an Area-Wide Integrated Pest Management (AW-IPM) programme to induce widespread population suppression. The successful implementation of the SIT programme required a series of well-researched phases, with one key phase being the determination of an effective overflooding ratio, previously established at 10:1. Despite this, various studies of *T. leucotreta* SIT programmes have reported higher release ratios than 10:1 in orchards, necessitating an investigation into their efficacy. This thesis aimed to understand the effects of higher release ratios, different combinations of sterile and fertile *T. leucotreta* of both sexes, compatibility of SIT and the egg parasitoid *Trichogrammatoidea cryptophlebiae*, and the pre-release mating levels during production and release stages to improve the effectiveness of the *T. leucotreta* SIT programme.

This study initially investigated the impact of different ratios of sterile and fertile adults on fruit damage, sterile male competitiveness, and population growth in laboratory cages using Washington Navel oranges. Sterilised to fertile *T. leucotreta* adults at ratios of 0:1 (control), 10:1, 20:1, 40:1, and 60:1 were placed inside insect-rearing cages and allowed to mate, oviposit and infest the fruit. Treatment cages receiving sterile *T. leucotreta* produced significantly fewer damaged fruit, larval entries, and F1 adults compared to the control. The number of damaged fruit, larval entries, and F1 adults negatively correlated with the increase in the overflooding ratio of sterile to fertile *T. leucotreta*. Control cages had significantly higher fecundity and fertility compared to treatment cages. The 60:1 ratio exhibited the lowest per generation rate of increase (<1× from the parental [P1] to the F1 generation) compared to the 10:1 ratio (current release ratio).

The effects of different combinations of both treated (T) and untreated (U) male (M) and female (F) adult *T. leucotreta*: UM×UF (control), TM×UF, UM×TF, TM×TF, and UM×UF×TM×TF on fruit damage, mating competitiveness, and per-generation rate of increase were tested. The treatments were housed in insect-rearing cages containing Navel oranges and allowed to mate, oviposit, and infest the fruit. Treatment cages with both treated male and female *T. leucotreta* had significantly fewer damaged fruit, larval entries, and emerged F1 adults compared to the control cages, except for the UM×UF×TM×TF combination. Similarly, control cages and UM×UF×TM×TF treatments had significantly

higher fecundity and fertility compared to other treatments. The TM×UF combination exhibited the lowest rate of increase per generation (<0.57× from the parental [P1] to F1 generation).

A field cage study was conducted to evaluate the effects of various overflooding ratios and different combinations of sterile and fertile male and female *T. leucotreta*. However, the results were limited, and inconclusive due to collection of insufficient data, as the fruit infestation level was low. This could be attributed to the low quality of the moths released or effects of environmental variables on the moths.

A laboratory study explored the susceptibility of *T. leucotreta* eggs resulting from various pairings of sterile and fertile moths to parasitism by *Trichogrammatoidea cryptophlebiae*. The ratios of sterile to fertile *T. leucotreta* used were: 0:1, 10:1, 20:1, 40:1, and 60:1. The resulting eggs were then exposed to *T. cryptophlebiae* for parasitism, and the parasitism rates of newly laid (24 h), 48 h and 72 h old eggs were evaluated. Overall, eggs from all ratios were suitable for *T. cryptophlebiae* development and acceptable for oviposition. Significantly higher number of parasitised eggs were recorded between the control (0:1) and ratios 40:1 and 60:1 at 48 h old eggs. Additionally, a higher proportion of flying *T. cryptophlebiae* emerged across the ratios, with a higher proportion of female-to-male sex ratio.

Pre-release mating levels were studied during the production and release stages, divided into three stages: moth eclosion, irradiation, and release. A significantly higher number of spermatophores and percentages of mated female *T. leucotreta* were recorded at the eclosion and irradiation stages in January. Similarly, in May, a significantly higher number of spermatophores and percentages of mated female *T. leucotreta* were recoded post-irradiation and release stages in the Sundays River Valley region (SRV). Overall results indicated more spermatophores and percentages of mated female *T. leucotreta* at the irradiation and release stages in the SRV region.

In conclusion, the study demonstrated that a release ratio exceeding 40:1 and different combinations of sterile and fertile *T. leucotreta*, especially the TM×UF combination, has a suppressive effect against *T. leucotreta* and integrating SIT with *T. cryptophlebiae*, shows potential for enhancing the effectiveness of the *T. leucotreta* SIT programme. Additionally, the mating competitiveness of sterile insects in dual-sex releases can be improved by controlling the level of pre-release matings.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Origin and distribution of citrus

The origin of the *Citrus* species is believed to be in the area spanning from south-eastern Asia, eastern Arabia, South of the Himalayas to Indonesia and Australia (Liu et al., 2012; Talon et al., 2020). This region is a biodiversity hotspot as the climate is tropical and greatly influenced by the eastern and southern Asian monsoons (Wu et al*.,* 2018; Talon et al., 2020). Wu et al. (2018) postulated that the south-eastern slopes of the Himalayas would have been the probable region for the origin of most citrus. Citrus, such as the limes, are thought to have originated in tropical areas, while oranges and mandarins evolved in more northerly or cooler mountainous areas within the region (Liu et al., 2012; Aidoo, 2019).

Cultivated citrus was introduced to the Americas between 1655 and 1769 by Spanish and Portuguese explorers (Liu et al., 2012; Aidoo, 2019) and then subsequently to the rest of the world. Currently, the main commercial production zones are the northern and southern Mediterranean, North and South America, and their associated islands, China, India, Japan, Australia, and South Africa (Liu et al., 2012). In Africa, citrus was discovered on St Helena Island by Juan de Nova Castella (a Portuguese explorer) in 1502 when heading home from India. It was supposedly introduced to the island by sailors, as it was commonly used in ships for the prevention and cure of scurvy (Aidoo, 2019). Citrus has been cultivated for over four centuries in nearly all countries within the latitudes ranging from 40°N to 40°S, (Kilalo, 2004; Liu et al., 2012).

1.2 Taxonomy and morphology

Citrus species are members of the Order Sapindales, family Rutaceae, and subfamily Aurantioideae, with the genus *Citrus* being its most prominent representative (Lv et al., 2015; Zech-Matterne & Fiorentino, 2017; Inglese & Sortino, 2019). The Aurantioideae is subdivided into two tribes, namely: Clauseneae, which contains five genera, and Citreae, which contains about 28 genera. The latter tribe consists of the genus *Citrus* and related genera such as *Fortunella*, *Poncirus*, *Eromecitrus*, *Microcitrus,* and *Clymenia*. The taxonomy of the genus *Citrus* is, hitherto, sophisticated, controversial, and sometimes confusing, due to numerous sexual compatibilities, the partial apomixis between *Citrus* species and related genera, and the long history of Citrus cultivation (Zech-matterne & Fiorentino, 2017).

Currently, there are two widely accepted taxonomic systems; Swingle's (1943) classification identifies 16 species, and Tanaka's (1977) system postulated 156 species (Lv et al., 2015; Zech-matterne & Fiorentino, 2017; Inglese & Sortino, 2019, Talon et al., 2020). Most horticultural citrus types such as the sweet orange (*Citrus sinensis* (L.) Osbeck.),

mandarin (*C. reticulata* Blanco), lemon (*C. limon* (L.) Burm.), grapefruit (*C. paradisi* Mac.), lime (*C. aurantifolia* (Christm.) Swing.) and pummelo (*C. maxima* (Burm.) Merr.), are each considered as species in Swingle's systematics. However, Swingle identified only one species of sweet orange (*C. sinensis*) while Tanaka described 12 species for this citrus horticultural type alone (Table 1.1) (Lv et al., 2015; Zech-matterne & Fiorentino, 2017; Inglese & Sortino, 2019). The classification of *Citrus* taxa continues to evolve as the genetic studies provide deeper insights into their phylogeny and diversity.

Table 1.1: Comparison of sweet orange taxonomy between Swingle's and Tanaka's systems.

Swingle (1943)	**Tanaka (1977)**
C. sinensis	*C. sinensis* (L) Osbeck
C. sinensis	*C. tankan* Tanaka
C. sinensis	*C. temple* Hort. ex Tan.
C. sinensis	*C. oblonga* Hort. ex Tan.
C. sinensis	*C. funadoko* Hort. ex Tan.
C. sinensis	*C. iyo* Hort. ex Tan.
C. sinensis	*C. sinograndis* Hort. ex Tan.
C. sinensis	*C. luteo-turgida* Tanaka
C. sinensis	*C. ujukitsu* Hort. ex
C. sinensis	*C. tamurana* Hort. ex Tan.
C. sinensis	*C. aurea* Hort. ex Tan.
C. sinensis	*C. shunkokan* Hort. ex Tan.

1.3 Citrus production in South Africa

The production of *Citrus* is the largest fruit industry in South Africa, mainly for the export market. In the southern hemisphere, South Africa is the largest citrus-fruit exporter, accounting for more than 60% of southern hemisphere citrus exports (Chisoro-dube &

Roberts, 2021). Globally, South Africa is the 13th largest citrus producer and the second largest citrus-exporting country, after Spain, accounting for 10% of global exports (Moore & Marankhan, 2022; Citrus Growers Association (CGA), 2023). Exports are the major source of revenue generation, where two-thirds of citrus produced is exported as fresh fruit (Sikuka, 2021; Chisoro-dube & Roberts, 2021). This accounts for about 95% of the total revenue generated by the industry per annum and provides lucrative employment opportunities for many stakeholders along the value chain. The main citrus production zones in Southern Africa are Limpopo, Eastern Cape, Western Cape, Mpumalanga, KwaZulu-Natal, Northern Cape, Northwest, Free State Provinces, Zimbabwe, and Eswatini (Fig. 1.1) (Statistics, 2020; Edmonds, 2021; Sikuka, 2021; Chisoro-dube & Roberts, 2021; CGA, 2022).

Limpopo is the largest citrus-producing province, accounting for 39% of the total area cultivated, followed by the Eastern Cape (27%), Western Cape (19%), and Mpumalanga (8%), and the other regions together account for 7% (Statistics, 2020; Edmonds, 2021; Sikuka, 2021; CGA, 2023) (Fig. 1.1). Climatic conditions in these regions differ, which leads to the production of different citrus varieties that are available at different periods across the year (Edmonds, 2021) (Table 1.2). Climatic conditions in the Eastern Cape and Western Cape are cooler and hence are suitable for the production of Navel oranges, lemons, limes, and tangerines/mandarins (soft citrus). The warmer climatic conditions in Mpumalanga, Limpopo, and KwaZulu-Natal provinces are best suited for the production of grapefruits and Valencia oranges (Edmonds, 2021; Sikuka, 2021) (Table 1.3).

In South Africa, oranges are the most produced citrus type, accounting for about 48% of the total area under citrus cultivation. There has been a significant increase since 2000 in the total area planted with soft citrus and lemons/limes, which is promoted by high investment returns, profit margins from soft citrus and lemon production (although no longer for the latter), and increased global demand (Statistics, 2020; Edmonds, 2021).

Fig. 1.1: Citrus growing areas in South Africa (CGA, 2023).

Table 1.2: Citrus varieties grown commercially in South Africa (CGA, 2023).

Citrus Type	Varieties
Grapefruits	Star Ruby, Marsh, Rose, Flame, Nelspruit Ruby (Nelruby), Flamingo
Oranges	**Valencia -** Delta, Midknight, Turkey, Oukloon (Olinda, Late), Du Roi, Benny.
	Navel - Palmer, Bahianinha, Washington, Robyn, Navelina, Lane Late, Newhall, Cambria, Cara Cara, Rustenburg, Autumn Gold
Mandarins/	**Clementine -** Nules, Marisol, SRA, Oroval, Esbal, Clemenpons, Oronules.
Tangerines	**Mandarin –** Tango, Nadorcott, Nova, Or (Orri), Minneola, Mor,
	B17, Tambor, Naartjie, Thoro Temple, Sonet, B24 (African Sunset)
	Satsuma - Miho Wase, Owari, Kuno, Miyagawa Wase, Okitsu Wase, Aoshima.
Lemons/Limes	Eureka, Eureka SL, Lisbon, Limoneira, Genoa

Table 1.3: South African harvest periods for different types of citrus (CGA, 2023).

Citrus	Harvest Period
Marsh Grapefruits	March to June
Star Ruby Grapefruits	April to September
Navel Oranges	March to July
Valencia Oranges	July to September
Mandarins/Tangerines	March to August
Lemons/Limes	February to September

1.4 Pests associated with citrus

Citrus species are susceptible to innumerable pests that can severely minimize production and even decimate the citrus industry of a country (Tennant et al., 2009). The degree of economic importance of a pest is determined by the extent and the type of damage caused. Climatic conditions such as humidity, temperature, and rainfall greatly affect citrus pests, where certain pests only gain pest status at certain times of the year when the conditions are conducive for their establishment (Tennant et al., 2009). Additionally, the pest status is also affected by the variety of citrus grown. For instance, although the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is regarded as a major pest of citrus, however, lemons and limes are regarded as non-hosts, particularly at the

commercially ripe stage for harvest (Smith & Peña, 2002; Love et al., 2014; Moore et al., 2015).

The classification of pests is based on the extent of damage they cause, the frequency of their damage, and the citrus plant parts attacked (Smith & Peña, 2002). Pests are classified as key pests when they occur frequently throughout the season and cause significant damage to the plant, whereas minor pests occur in small numbers, are easily controlled, and cause trivial damage (Smith & Peña, 2002; Kilalo, 2004). Some citrus pests, such as certain scale insects (Hemiptera: Coccomorpha: Diaspididae), may be referred to as secondary pests since, under natural conditions, the pest population is usually low due to control by natural enemies. However, if these natural control agents are disturbed, for instance, through chemical control, the pest populations will increase (Smith & Peña, 2002).

The damage inflicted by a pest may be directly on the fruit, through the larvae feeding on the developing fruit, making it rot or premature drop from the tree, which compromises the quality and quantity of the fruit produced. Pests can also cause indirect damage by being vectors of plant pathogens. For instance, Asian citrus psyllid, *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllomorpha: Liviidae) and African citrus psyllid, *Trioza erytreae* (Del Guercio) (Psyllomorpha: Triozidae), transmit the bacterium *Candidatus* Liberibacter *asiaticus* and *Candidatus* Liberibacter *africanus*, the causal agents of Asian citrus greening and African citrus greening diseases, respectively. These diseases cause severe stunting and leaf mottling in citrus trees (Rwomushana et al., 2017; Urbaneja, 2020). Some other insect pests such as citrus thrips, mites, leafrollers, African bollworms, and some mealybugs, cause cosmetic damage by affecting the appearance of the fruit (Annecke & Moran, 1982; Urbaneja, 2020). Due to consumer demand, blemished fruit are usually considered unsuitable for export markets, resulting in losses to citrus growers (Smith & Peña, 2002; Kilalo, 2004; Urbaneja, 2020).

Globally, citrus is attacked by an arthropod complex of about 875 species of insects and mites; however, only 144 of these are recognised as key pest species (Smith & Peña, 2002). In South Africa, about 110 citrus pest species are known to be of economic importance (Bedford, 1998). The arthropod complex greatly affecting citrus in South Africa belongs to the orders Thysanoptera, Hemiptera, Lepidoptera, Diptera, and Trombidiformes (Ryke & Meyer, 1960; Bedford, 1998; Smith & Peña, 2002; Grout & Moore, 2015). The key pests include citrus thrips, *Scirtothrips aurantii* (Faure) (Thysanoptera: Thripidae); California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae); citrus psyllid, *T. erytreae* (Del Guercio) (Hemiptera: Triozidae); citrus mealybug, *Planococcus citri* (Risso)

(Hemiptera: Pseudococcidae); Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae); Natal fruit fly, *C. rosa* (Karsch) (Diptera: Tephritidae); and false codling moth, *T. leucotreta*, the target species of this study (Table 1.4) (Smith & Peña, 2002; Grout & Moore, 2015). Citrus is also attacked by several minor or sporadically important pests such as some orthopterans, snails, slugs, soft scales, nematodes, mites, and ants (Ryke & Meyer, 1960; Annecke & Moran, 1982).

Table 1.4: Herbivory feeding guilds of citrus pests in South Africa, including some of their pests (Ryke & Meyer, 1960; Bedford, 1998; Moore, 2003; Grout & Moore, 2015).

Feeding Guilds	**Common name**	**Scientific name**	**Plant parts attacked**	**Damage**
Leaf and flower chewers	**Shield bugs**	**Family Pentatomidae**		
	Antestia bug	*Antestiopsis thunbergii* (Gmelin)	Blossoms and fruit	Blemishes on fruit resulting in fruit drop in severe situation
	Snout beetles	**Family Curculionidae**		
	Fuller's rose beetle	*Pantomorus cervinus* (Boheman)	Leaves	Leaf notching
	Pyralid snout moths	**Family Pyralidae**		
	Citrus flower moth	*Prays citri* (Milliere)	Blossoms	Flower feeding by larvae resulting in quick wilt and drop
	Loopers	**Family Geometridae**		
	Citrus looper	*Ascotis reciprocaria* (Walker)	Leaves and fruitlets	Causes superficial damage on the top and bottom of young leaves
	Swallowtails	**Family Papilionoidea**		
	Citrus swallowtail	*Papilio demodocus* (Esper)	Leaves	Leaves feeding resulting in

				defoliation
Sap suckers	**Hard scales**	**Family Diaspididae**		
	Mussel scale	*Lepidosaphes beckii* (Newman)	Leaves, twigs, and fruit	Leaf chlorosis, defoliation, twig dieback, fruit drop, and fruit poch-marking
	Long mussel scale	*Lepidosaphes gloverii* (Packard)		
	Circular purple scale	*Chrysomphalus aonidum* (Linnaeus)	Leaves and fruit	Defoliation, twig dieback, fruit poch-marking
	Red scale	*Aonidiella aurantii* (Maskell)		
	Soft scales	**Family Coccidae**		
	White wax scale	*Ceroplastes brevicauda* (Hall)	Leaves and twigs	Formation of sooty mould resulting in fruit staining
	Citrus wax scale	*Ceroplastes destructor* (Newstead)		
	Brown soft scale	*Coccus hesperidum* (Linnaeus)		
	Green soft scale	*Pulvinaria aethiopica* (De Lotto)	Leaves	Sooty mould formation resulting in fruit staining
	White powdery scale	*Pseudocribrolecanium andersoni* (Newstead)		
	Giant coccids	**Monophlebidae**		
	Cottony cushion scale	*Icerya purchasi (*Maskell)	Leaves and twigs	Sooty mould formation, twig dieback, leaf, and fruit drop
	Mealybugs	**Pseudococcidae**		
	Citrus mealybug	*Planococcus citri (*Risso)	Leaves, twigs, and fruit	Formation of dents and lumpy shoulders in the
	Oleander	*Paracoccus burnerae*		

	mealybug	(Brain)		fruit tissue, curling of leaves, sooty mould formation resulting in fruit staining
	Karoo thorn mealybug	*Nipaecoccus viridis* (Newstead)		
	Long-tailed mealybug	*Pseudococcus longispinus* (Targioni Tozzetti)		
	Aphids	**Family Aphididae**		
	Black citrus aphid	*Toxoptera aurantii* (Boyer de Fonscolombe)	Leaves, twigs, and fruit	Leaf buckling, stunted growth, blossom drop, sooty mould formation. Vectors for Citrus Tristeza Virus (CTV)
	Spirea aphid	*Aphis spiraecola* (Patch)		
	Thrips	**Family Thripidae**		
	Citrus thrips	*Scirtothrips aurantii* (Faure)	Leaves and fruit	Fruit scarring around the calyx
Gall makers	**Citrus triozid**	**Family Triozidae**		
	Citrus triozid	*Trioza erytreae* (Del Guercio)	Leaves	Formation of leaf galls. Vector for Candidatus Liberibacter *africanus*
Leaf miners	**Leaf miner moths**	**Family Gracillariidae**		
	Citrus leaf miner	*Phyllocnistis citrella* (Stainton)	Leaves and twigs	Serpentine mines on leaves by larvae resulting in leaf curl and leaf yellowing.
Fruit	**Fruit flies**	**Family Tephritidae**		

feeders	Mediterranean fruit fly	*Ceratitis capitata* (Wiedemann)	Fruit	Oviposition in ripening fruit resulting in fruit drop and rot
	Natal fruit fly	*Ceratitis rosa* (Karsch)		
	Leaf roller moths	**Family Tortricidae**		
	Apple leaf roller	*Lozotaenia capensana* (Walker)	Fruit and leaves	Blemishes on the fruits. Larvae feed on foliage
	Citrus leaf roller	*Choristoneura occidentalis* (Walshingham)	Fruit	Fruit burrowing by larvae resulting in fruit drop
	False codling moth	***Thaumatotibia leucotreta*** (Meyrick)		
	Pyralid snout moths	**Family Pyralidae**		
	Carob moth	*Ectomyelois ceratoniae* (Zeller)	Fruit	Fruit burrowing by larvae resulting in fruit drop
	Owlet moths	**Family Noctuidae**		
	African bollworm	*Helicoverpa armigera* (Hübner)	Fruitlets and leaves	Feeding on fruitlets and leaves by larvae. Causes deep holes in fruits
	Fruit-piercing moth	*Serrodes partita* (Fabricius)	Fruit	Fruit piercing, sucking up juices causing fruit drop after fungi infection
	Red spider mites	**Family Tetranychiidae**		
	Citrus red mites	*Panonychus citri* (McGregor) *Eutetranychus banksi* (McGregor)	Fruit, sometimes on leaves	Fruit scarring, silvering of the leaves

	False spider mites	*Brevipalpus phoenicis* (Geijskes)		
	Gray mites	**Family Eriophyidae**		
	Citrus gray mites	*Calacarus citrifolli* (Keifer)	Fruit	Causes concentric ring blotch of citrus fruit
Others	**Ants**	**Family Formicidae**		
	Pugnacious ants	*Anoplelopis custodiens* (Smith)		Causes no direct damage to citrus plants but tends to defend honeydew-producing pests of citrus from their natural enemies and sometimes transport their immature stages to new foliage
	Brown house ants	*Pheidole megacephala* (Fabricius)		
	Snails	**Family Achitinidae**		
	Large land snail	*Achatina immaculata* (Larmack)	Fruit and leaves	Bores holes into the leaves and fruits.
	Brown snail	*Helix aspersa* (Müller)		
	Dune snail	*Theba pisana* (Müller)		
	Slugs	**Family Urocyclidae**		
	Terrestrial slugs	*Urocyclus kirkii* (Gray)		
		Urocyclus flavescens (Kefer)		

1.5 The false codling moth, *Thaumatotibia leucotreta*

1.5.1 Pest taxonomy and geographic distribution

Thaumatotibia leucotreta was reported by Fuller (1901) as a pest of citrus in KwaZulu-Natal (Moore, 2002; Daniel, 2016). Fuller (1901) identified it as the Natal codling moth due to it having the same appearance, behaviour, and development and causing the same sought of damage as the true codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), except that citrus fruit were attacked, rather than apples. Howard (1909) also mistakenly recorded it as an orange codling moth, *Enarmonia batrachopa* (Meyrick). He later, Meyrick (1913) described it as *Argyroploce leucotreta* (Stotter, 2009), but Kelly (1914) described it as *Enarmonia batrachopa* (Meyrick) after finding it on acorns near Pietermaritzburg. In 1958, *T. leucotreta* was transferred by Clarke to the genus *Cryptophlebia*, as *Cryptophlebia leucotreta* (Clarke, 1958). Komai (1999) transferred it from *Cryptophlebia* to the genus, *Thaumatotibia* as *Thaumatotibia leucotreta* Meyrick (Stotter, 2009; Timm & Brown, 2014). In South Africa, *T. leucotreta* may be confused with a few other insect species, such as the true codling moth, *C. pomonella*, macadamia nut borer, *Thaumatotibia batrachopa* (Lower) (Lepidoptera: Tortricidae) and litchi moth, *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) (Venette et al., 2003; Stibick, 2006; Stotter, 2009).

Thaumatotibia leucotreta is an endemic pest to sub-Saharan Africa (SSA) and African islands (Gilligan et al., 2011; European and Mediterranean Plant Protection Organization (EPPO), 2013; Adom et al., 2021; European Food Safety Authority (EFSA), 2024). It is mostly found in areas that are classified as deserts, tropical and subtropical regions, savannah grasslands, and rainforests (Venette et al., 2003; Adom et al., 2021). The pest was reported in Israel for the first time in 1984 on macadamia nuts and was considered to have a limited distribution there (Wysoki, 1986; EPPO, 2002; EPPO, 2013; EFSA, 2024). In Africa, it is thought that *T. leucotreta* originated in the Ethiopian region, but it is now widely distributed, having been reported in 36 African countries (Fig. 1.2), including some islands (Centre for Agriculture and Bioscience International (CABI), 2000; Adom et al., 2021). *Thaumatotibia leucotreta* is well established in the following African countries: Angola, Benin, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Côte d'Ivoire, Democratic Republic of Congo, Eritrea, Ethiopia, Gambia, Ghana, Kenya, Madagascar, Malawi, Mali, Mauritius, Mozambique, Niger, Nigeria, Rwanda, Réunion, Saint Helena, Senegal, Sierra Leone, Somalia, South Africa, Sudan, Eswatini, Tanzania, Togo, Uganda,

Zambia, and Zimbabwe (Stibick, 2006; EPPO, 2013; Daniel, 2016; EPPO, 2024). However, among the African countries where the pest has been reported, only in South Africa has there been extensive research on the pest. This is crucial for making informed decisions and addressing the strict phytosanitary measures put in place by export markets since South Africa is the second-largest citrus exporter globally (Adom et al., 2021).

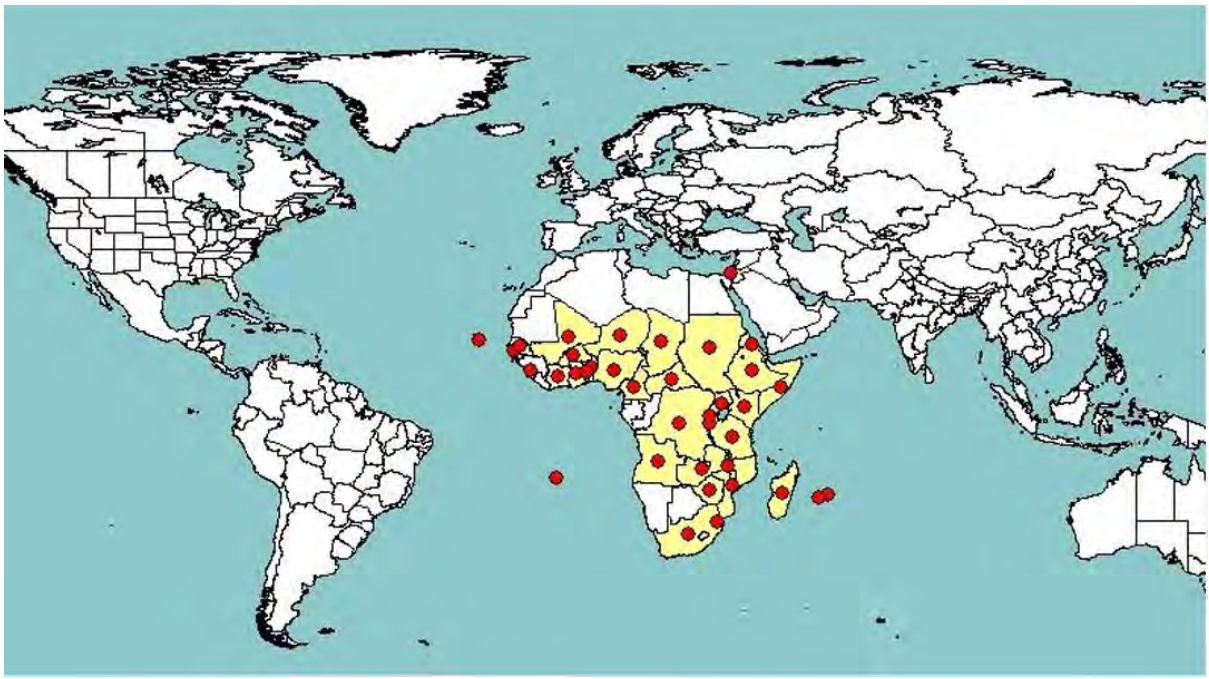

Fig. 1.2: Distribution of *T. leucotreta* in Africa and neighbouring islands (EPPO, 2024) https://gd.eppo.int/taxon/ARGPLE/distribution (Accessed 2024-07-08). Red dots indicate *T. leucotreta* presence.

1.5.2 Biology of *T. leucotreta*

Thaumatotibia leucotreta is a holometabolous insect that undergoes four developmental stages namely, egg, larva (five instars), pupa, and adult (Stibick, 2006; Loomans et al., 2020; EFSA, 2024); the life cycle has no diapause stage (Reed, 1974; Stibick, 2006; Terblanche et al., 2014) and takes between 35 and 174 days to complete, depending on climatic conditions (Fig. 1.3) (Stibick, 2006; de Jager, 2013). Under optimum temperature (25°C), it takes an average of 42-46 days (Opoku-Debrah et al., 2014). *Thaumatotibia leucotreta* can remain active throughout the year, provided the right host plant is available. On citrus, the pest has been reported to have 5-6 generations per year in South Africa (Venette et al., 2003; EFSA, 2024). Certain factors such as food availability/quality, temperature, diseases, predators, photoperiods, and moisture levels, affect the number of generations per year (Venette et al., 2003). Infestation levels drop during high rainfall periods

as high soil moisture levels result in anaerobic conditions fatal to the pupae and favour the development of entomopathogenic fungi and nematodes that kill the pupae, thus reducing the pest population (Daiber, 1979). *Thaumatotibia leucotreta* females tend to live longer than males, with a ratio of 1:1 wild females to males (Daiber, 1979).

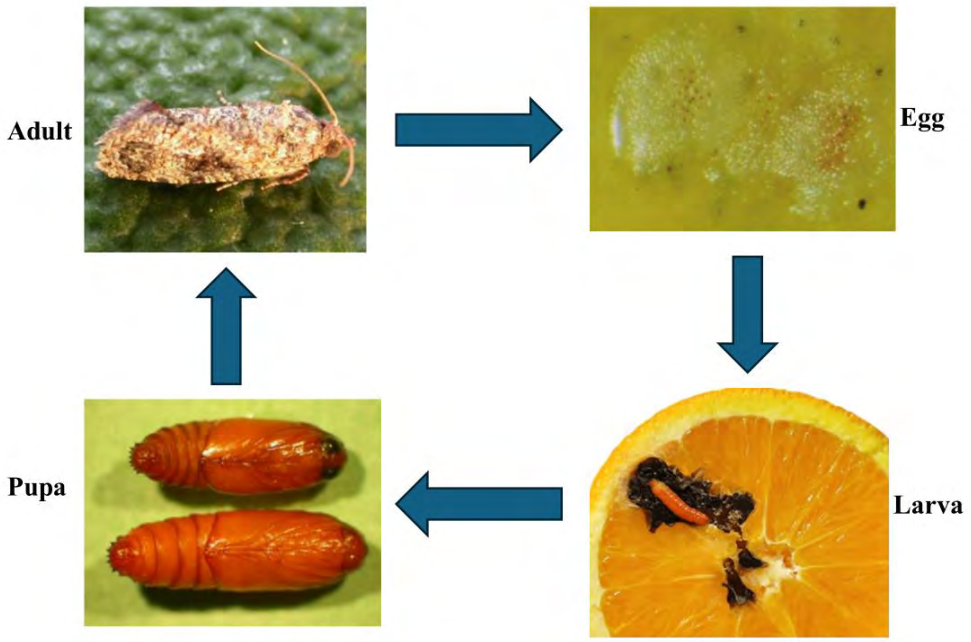

Fig. 1.3: The life cycle of *T. leucotreta*.

1.5.2.1 Egg stage

Thaumatotibia leucotreta is nocturnal; fertile females fly during the night ovipositing their eggs on plant surfaces from around sunset to the early morning hours (Daiber, 1980; Venette et al., 2003; Stibick, 2006; de Jager, 2013; Loomans et al., 2020). The female starts laying eggs after 2-3 days of emergence from the pupa (Daiber, 1980). Oviposition on physically damaged and early-ripening fruit is much greater than on healthy fruit in a normal stage of development (Newton, 1998). The eggs are laid at random, singly on the fruit rind/bolls/nuts of the host plants (Daniel, 2016; Loomans et al., 2020) (Fig. 1.4A). Each female can lay about 100 eggs, but when temperatures are favourable, the number can reach 800, with peak egg-laying taking place over 3-4 days depending on the host species or the diet (Newton, 1998; Daiber, 1980; Loomans et al., 2020; Adom et al., 2021). Each egg is a flat, ovoid with a granulated surface, white to cream in colour when laid (Fig 1.4A) but changing to red or pink as development progresses (Fig. 1.4B) until a larva with a black-

headed capsule is formed, ready for hatching (Daiber, 1980). Eggs take 2-22 days to develop and are temperature- and humidity-sensitive (Adom et al., 2021). There is a direct relationship between the time taken for the egg to hatch and the temperature, as high temperatures and humidity are correlated with the high rate of egg development (Daiber, 1980). De Jager (2013) reported egg incubation periods of 12.42, 9.46, and 6.47 days on orange, grape, and pear, respectively.

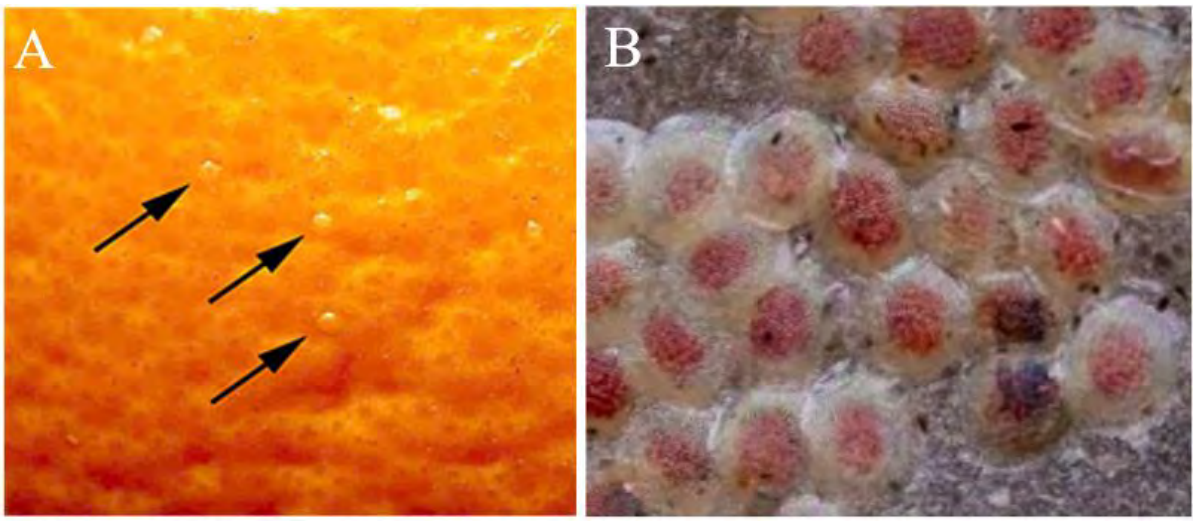

Fig. 1.4: Freshly laid translucent eggs of *T. leucotreta* on an orange rind **A**; developing (red/pink) eggs of *T. leucotreta* **B**.

1.5.2.2 Larval stage

Hatching occurs at all times during the day (Daiber, 1979). After hatching from the eggs, the newly emerged neonate larvae burrow into the fruit, each making a hole about 1 mm in diameter. The larvae are also opportunistic and sometimes gain access to the fruit through wounds and cracks of secondary infestations (Stotter, 2009; de Jager, 2013; Adom et al., 2021). The point of entry becomes conspicuous due to the excreta (frass) left on the surface and the discolouration of the infested rind (Daiber, 1980; Stibick, 2006). The 1 mm-long neonates have a dark pinacula with a spotted appearance, while the fifth-instar larvae are 15 mm long, orange-pink with a brown head capsule and prothoracic shield (Fig. 5). The development of the larva takes 12-33 days during warm conditions, compared to 35-67 days in cooler conditions (Daiber, 1979). The development time of the larvae may be affected by the nutritional quality of the fruit (Daiber, 1979; Stibick, 2006). Once the larvae gain entry, they feed on the pulp, and as they develop, they move towards the centre of the fruit (Fig. 1.5). Mostly, the survival rate per fruit is only one larva, as they are cannibalistic, but in some

instances, a maximum of three per fruit has been reported (de Jager, 2013; Adom et al., 2021). Once the larva reaches maturity the fruit may have dropped to the ground (Fig. 1.8B). However, if the fruit is still attached to the plant, the larva forms a silken thread aiding it to drop to the topsoil or leaf litter, where it pupates beneath the surface (Stibick, 2006; Adom et al., 2021). The average head capsule widths of the 1st, 2nd, 3rd, 4th, and 5th larval instars are 0.21, 0.37, 0.61, 0.94, and 1.37 mm, respectively (Daiber, 1979).

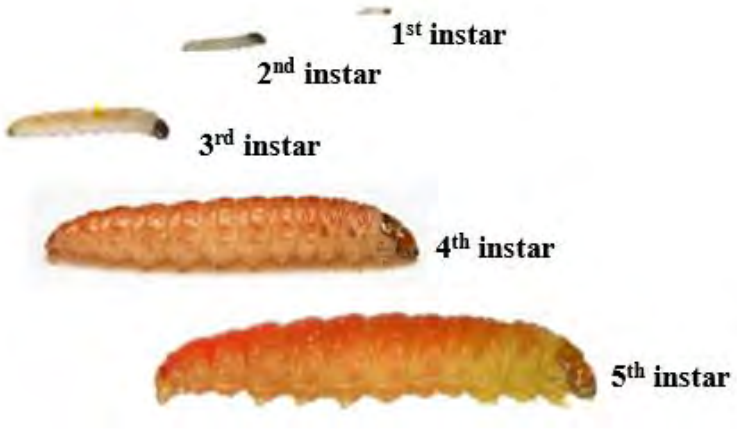

Fig. 1.5: *Thaumatotibia leucotreta* larval instars (not according to aforementioned sizes) (David Taylor, CBC).

1.5.2.3 Pupal stage

Once the mature fifth-instar drops into the soil, it spins a cocoon made of soil and some silky body secretions (Daiber, 1980). The cocoon is white to cream-coloured. The time taken for pupal development is dependent on gender and the temperature, whereby there is a high emergence during high temperatures compared to a lower emergence rate during cooler periods. It takes from 13-49 days for the males to reach maturity, while the females take from 11-39 days (Daiber, 1980). When young, the pupae are sensitive to low temperatures and rainfall (Daiber, 1979). The pupal sex ratio in a wild moth population was reported to be 1:1 males to wild females (Myburgh & Bass, 1969; Daiber, 1979). Each pupa (Fig. 1.6) develops into an adult moth within the pupal casing, and then eclosion takes place (Daiber, 1979; de Jager, 2013; Adom et al., 2021).

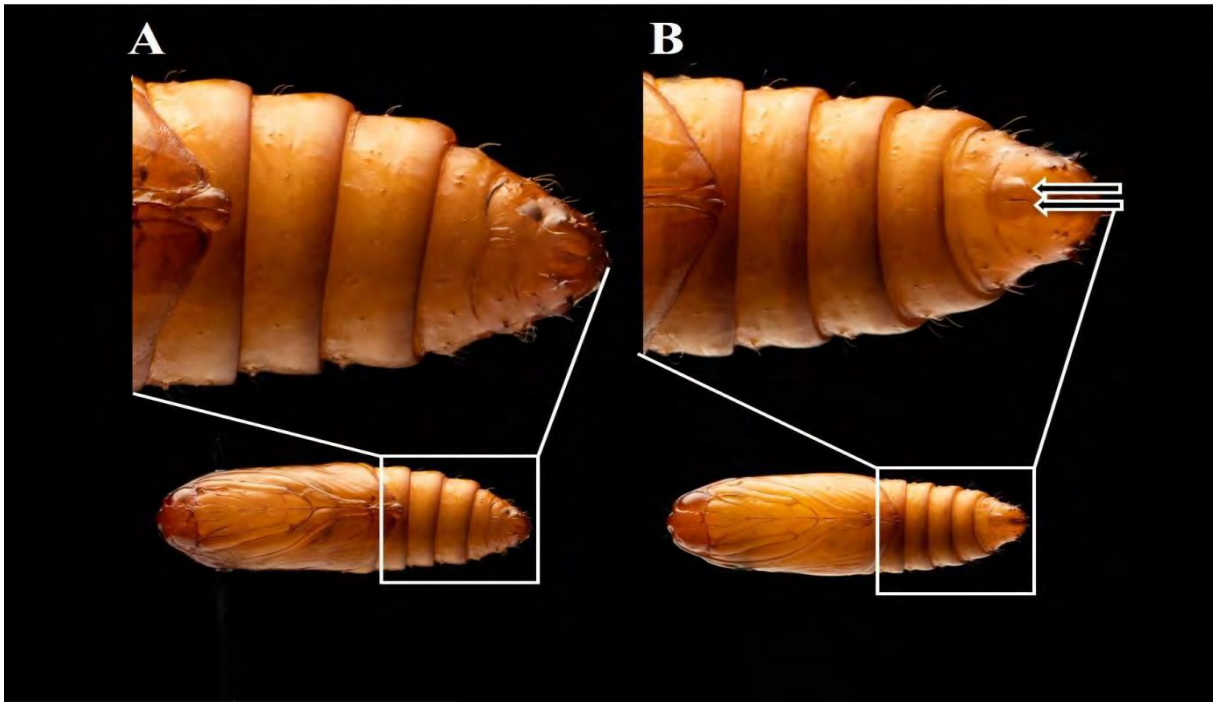

Fig. 1.6: *Thaumatotibia leucotreta* pupae: Left (A), female; Right (B), male (David Taylor, CBC). Features that distinguish female and male *T. leucotreta* pupae, (A) female with 4 abdominal segments, lacking two knobs on the ventral side of the final segment, and (B) males with 5 abdominal segments with two knobs on the ventral side of the final segment. Arrows show the two knobs present in male *T. leucotreta* pupae (Daiber, 1979).

1.5.2.4 Adult stage

Thaumatotibia leucotreta adult moths are nocturnal, inactive, resting under the shade of host plants during the daytime and flying at night (Venette et al., 2003). The moths are small and inconspicuous, having a colour variation of grey to dark brown. Males have an average forewing length of 7-8 mm compared to 9-10 mm for females (Fig. 1.7). The males are differentiated from the females by having a big, greyish genital tuft; long, dense scales on the hind legs; and the hind tibia being heavily tufted (Venette et al., 2003; Gilligan et al., 2011). The semi-circular pocket of opalescent scales on the distal end of vein CuA2 of the hindwing is a useful feature used to also distinguish the male *T. leucotreta* from the female. This characteristic also helps to distinguish *T. leucotreta* males from other tortricids found in North America (Gilligan et al., 2011). The forewing costal fold is absent in the male.

The lifespan of the male *T. leucotreta* is shorter than that of the female, with the latter living 16-70 days compared to the males that live for 15-57 days (Daiber, 1980). The availability of host plants and temperature determine the dispersal range of the adult moth

(Stibick, 2006). The males are attracted to the females along a plume of volatile pheromones shortly after darkness. Pheromone release peak drastically after five hours and then decreases until sunrise (Stibick, 2006; Loomans et al., 2020). The life cycle of the moth is longest in low temperatures, but when conditions are conducive, can reach about six generations annually (Daiber, 1980).

Fig. 1.7: *Thaumatotibia leucotreta* adults: Left, female; Right, male with the arrow pointing to the androconial (scent organ) (P. R Stephen, Citrus Research International [CRI]).

1.5.3 Host plants of *T. leucotreta*

Thaumatotibia leucotreta is a polyphagous insect that has been reported to infest more than 130 plant species belonging to 51 families (Venette et al., 2003; Stibick, 2006; EPPO, 2013; Grové et al., 2014; Loomans et al., 2020; CABI, 2022; EFSA, 2024; EPPO, 2024). However, many of these host associations were recorded in the laboratory and it is uncertain whether the same would occur in the wild (Moore et al., 2015). Several of this reported associations are also unsubstantiated. Consequently, the current and most reliable host list consists of 107 plant species (EPPO, 2013; Moore et al., 2015; EPPO, 2024). Twenty cultivated plants have been reported as potential hosts for *T. leucotreta* in South Africa (Grové et al., 2014). In South Africa, the most preferred host is citrus, and the most highly attacked cultivars of citrus are Navel oranges and mandarins (Moore et al., 2016; EFSA, 2024). The wide host range of *T. leucotreta* (Table 1.5) enables its population to survive even in the absence of citrus (EFSA, 2024).

Table 1.5: Major cultivated crops recorded as hosts to *T. leucotreta* (CABI, 2022; EFSA 2024; EPPO, 2024).

Common name	Scientific name	Family
Navel orange	*Citrus sinensis*	Rutaceae
Olive	*Olea europaea*	Oleaceae
Avocado	*Persea americana*	Lauraceae
Grape	*Vitis vinifera*	Vitaceae
Peach	*Prunus persica*	Rosaceae
Pomegranate	*Punica granatum*	Punicaceae
Litchi	*Litchi chinensis*	Sapindaceae
Capsicum	*Capsicum* sp.	Solanaceae
Macadamia	*Macadamia integrifolia*	Proteaceae
Coffee	*Coffea arabica*	Rubiaceae
Cacao	*Theobroma cacao*	Malvaceae
Carambola	*Averrhoa carambola*	Oxalidaceae
Cotton	*Gossypium hirsutum*	Malvaceae
Roses	*Rosa* sp.	Rosaceae

1.5.4 Economic importance of *T. leucotreta*

The export of citrus is a multi-million Rand industry where South Africa is ranked as the second-largest citrus exporter globally after Spain, earning the country more than 30 billion South African Rand (ZAR) (2.05 billion USD) annually (CGA, 2023). *Thaumatotibia leucotreta* is associated with pre- and post-harvest fruit loss and rejection of exported consignments with fruit infestation signs. However, the losses incurred by citrus growers as a

result of the *T. leucotreta* attack are impossible to estimate due to the variation of damage from orchard to orchard and from season to season. Despite that, Moore et al. (2004) estimated the average pre-harvest losses from the pest to be about ZAR100 million (14 million USD) per annum in 2004 (outdated). Pre-harvest losses are due to burrowing and feeding of the larvae inside the fruit, which leads to fruit drop. Post-harvest losses occur due to fruit decay, resulting from secondary fungal and bacterial infections that gain access into the fruit once the protective rind integrity has been compromised, as well as phytosanitary rejections (Newton, 1998; Moore & Kirkman, 2008; Love, 2015). The burrows caused by *T. leucotreta* larvae are important entry points for pathogens, such as *Penicillium italicum* (Wehmer) and *P. digitatum* (Saccardo), the causative agents of citrus blue and green mould, respectively (Newton, 1998). Once the larva enters the fruit, it results in premature ripening, which leads to the early dropping of the fruit from the tree (Fig. 1.8B) (Love, 2015). Decayed and abscised fruit are unusable (Fig. 1.8A), resulting in a capital loss to both the farmers and the citrus industry.

Fig. 1.8: *Thaumatotibia leucotreta* larva in a Navel orange fruit **A**; fruit drop as a result of *T. leucotreta* infestation **B** (J.H. Hofmeyr, CRI).

With *T. leucotreta* being a regulated phytosanitary pest for most of South Africa's export markets, such as the EU, People's Republic of China, Republic of Korea, and the United States of America (USA), should any *T. leucotreta* be intercepted in a fruit shipment by any of these countries, the entire consignment is rejected, which results in a considerable economic loss (Hattingh et al., 2020; Moore, 2021). Consequently, the cold sterilisation approach (see 'cold treatment' in 1.6.5.6 Post-harvest control, below) has been adopted for the treatment of fruit during export to most phytosanitary markets (Hattingh et al., 2020;

Moore & Manrakhan, 2022). However, this is quite expensive, not only because of logistical and infrastructural challenges, but certain citrus types and cultivars are highly susceptible to cold treatment injuries, rendering them unsuitable for export under such a regime (Moore et al., 2016; Moore, 2021; Moore & Manrakhan, 2022). Consequently, it is essential to maintain orchards that are *T. leucotreta*-free, if at all possible, since its control below the economic injury level no longer applies.

1.5.5 *Thaumatotibia leucotreta* management

Several options are available for controlling *T. leucotreta* populations in citrus orchards in South Africa. Different control methods are combined to form an integrated pest management (IPM) control programme (Malan et al., 2018). *Thaumatotibia leucotreta* control can be successful in both pre- and post-harvest stages when the available control options are implemented properly. Before any control method is used, monitoring for *T. leucotreta* is recommended.

1.5.5.1 Monitoring

Monitoring is an essential step for the appropriate management of any agricultural pest. It helps to determine the pest abundance, risk potential, appropriate selection of control measures, the extent of damage, and the efficacy of the control methods used (Malan et al., 2018). The information obtained is useful when combined with any historical data, as it helps in giving time-series information on any future expected population pressure and determining what management option is needed (Loomans et al., 2020). Due to the cryptic nature of the eggs, they are difficult to locate. However, they become more conspicuous as their development progresses (Malan et al., 2018). The most important information is larval infestation and signs thereof. However, *T. leucotreta* larvae can be easily confused with some other fruit-attacking moth species, such as the codling moth or carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) (Malan et al., 2018), with only the latter being relevant to citrus.

Pheromone-baited traps are used during pre-harvest monitoring; males are attracted and caught in sticky traps using synthetic female pheromones as a lure, which aids in determining *T. leucotreta* population levels in orchards (Malan et al., 2018; Moore, 2022). Currently, three different types of pheromone-monitoring systems are registered, namely the Lorelei, FCM PheroLure, and Chempac FCM Lure (Moore, 2002; Albertyn, 2017; Moore, 2022). Pheromone-loaded dispensers are usually placed in yellow delta traps, with sticky

floors horizontally positioned at the bottom to trap any male lured in, to land on the glue. Lorelei was developed to help in determining the spray treatment threshold for *T. leucotreta*; the economic threshold was determined to be 10 or more moths per trap per week (Moore, 2002). However, with *T. leucotreta* gaining phytosanitary status in the export markets, the economic threshold no longer applies, as the pest is now of phytosanitary rather than economic concern, so should be completely controlled (Moore, 2022). According to Moore (2022), fruit drop surveying is one of the crucial methods for monitoring *T. leucotreta*. *Thaumatotibia leucotreta* population fluctuations can also be determined by scouting for eggs on the fruit. However, this is difficult due to the transparent nature of freshly laid eggs and their small size (Moore, 2022).

1.5.5.2 Cultural control

The main cultural control method practised by citrus farmers is orchard sanitation. It involves weekly removal of fallen fruit and damaged fruit found hanging on the trees, which helps to prevent population build-up in the orchard, as *T. leucotreta* larvae will survive in dropped fruit from citrus trees (Newton, 1998; Moore & Kirkman, 2008; Albertyn, 2017; Moore, 2022). Sanitation forms the foundation of *T. leucotreta* control in an orchard. The last-larval instars emerge from the fruit and pupate in the top layer of the soil or leaf litter. The number of pupae in the soil debris determines the size of the next *T. leucotreta* generation in the orchard. Removal and destruction of all dropped fruit helps to interrupt the life cycle, preventing *T. leucotreta* population build-up (Loomans et al., 2020). Weekly orchard sanitation of dropped fruits helps to remove 60-75% of *T. leucotreta* larvae (Moore & Kirkman, 2008; Moore, 2022). Additionally, this exercise should be conducted immediately after harvesting in the orchard is completed. The collected damaged fruit should be disposed of by burying them in a pit about 30 cm in depth or dunking fruit in water for 7 days. Alternatively, fruit can be mechanically destroyed using a hammer mill, although it is a tedious and time-consuming activity (Moore, 2022). All fruit left on the tree after harvest should also be removed to eliminate this bridge for the survival of the *T. leucotreta* population into the following season (Love, 2015).

Another cultural control method that can be employed by farmers is the use of citrus varieties that are less susceptible to pest attack (Adom et al., 2021). Different varieties of Navel oranges show variation in their level of *T. leucotreta* attractiveness and susceptibility (egg-laying and larval burrowing) (Love et al., 2014). Palmer Navels, which mature mid-season, are highly susceptible to *T. leucotreta* attacks compared to other Navel cultivars

(which mature mid-season), early maturing Fischer Navels, and Cambria and Glen Ora Late Navels, which are late-maturing varieties (Love et al., 2014).

1.5.5.3 Chemical control

Insecticides such as organophosphates, pyrethroids, and insect growth regulators with different modes of action have been used to control agricultural pests (Moore, 2002). Chemical insecticides have been shown to be effective in the control of *T. leucotreta*. However, there are several reasons why an alternative strategy needs to be used rather than chemical insecticides. The first reason is the ability of *T. leucotreta* to develop resistance to certain registered insecticides (Hofmeyr & Pringles, 1998). Secondly, stringent pesticide residue restrictions have been put in place by key export markets. Thirdly, insecticides can harm non-target organisms such as predators, parasitoids, and pollinators (Catling & Aschenborn, 1974; Moore, 2002). This not only can cause secondary pest resurgence but can also negatively compromise agroecosystem health (Malan et al., 2018).

Citrus Research International's (CRI) *T. leucotreta* management guidelines recommend the procedures for chemical control for *T. leucotreta* in South Africa (Moore, 2022). These guidelines list eight registered chemical insecticides available for *T. leucotreta* control (Moore, 2022): Alystin® (Triflumuron, Bayer CropScience South Africa (SA)); Nomolt® (Teflubenzuron, BASF SE Germany); Runner™ and several generics (Methoxyfenozide, Dow AgroSciences USA); Cypermethrin® (Cypermethrin, Agro-Serve (Pty) Ltd SA, amongst many); Meothrin® (Fenpropathrin, Philagro (Pty) Ltd SA); Delegate® (Spinetoram, Corteva AgriScience SA); Coragen® (Rynaxypyr, FMC AG USA); and Warlock® (Emamectin benzoate, ADAMA SA) (Moore, 2002; Moore, 2022). Different *T. leucotreta* developmental stages are targeted by different insecticides.

Due to the reasons given above, a more sustainable alternative chemical insecticide regime has to be used in South Africa, which is IPM compatible (Moore & Hattingh, 2012). This involves a combination of judicious control techniques that are effective and environmentally friendly to help improve citrus production. Such a programme has been implemented by CRI to curb phytosanitary pests like *T. leucotreta* in South Africa (Moore & Hattingh, 2012). When IPM control measures are implemented well, it results in a decrease in *T. leucotreta* infestation by 97% in the orchards (Smith & Peña, 2002; Moore et al., 2017).

1.5.5.4 Behavioural control

Attract and kill technique, mating disruption and sterile insect technique (SIT) are used as behavioural controls of *T. leucotreta* in South Africa (Malan et al., 2018; Moore, 2022). Both techniques involve the attraction of male *T. leucotreta* using synthetic female *T. leucotreta* pheromones. In the attract-and-kill technique, an insecticide-coupled pheromone is used to prevent mating, where males are killed once they come into contact with the insecticide, eliminating them permanently (Moore & Hattingh, 2012). There is only one registered attract-and-kill product, namely Last Call FCM (Insect Science (Pty) Ltd., Tzaneen, South Africa). It contains both synthetic pheromones and a pyrethroid insecticide encapsulated in a transparent gel. The attract-and-kill technique should only be used when the *T. leucotreta* population is low, mainly during the early season before population build-up (de Jager, 2013; Malan et al., 2018; Moore, 2022).

Mating disruption is an approach that relies on preventing mating between *T. leucotreta* individuals, consequently decreasing the number of fertile eggs laid on the fruit (de Jager, 2013; Malan et al., 2018; Moore, 2022). This is done by saturation use of a synthetic female pheromone to mislead or confuse the males; hence no mating or at least dramatically reduced mating occurs as males are unable to locate the females. However, the effect is temporary, and in the absence of synthetic pheromones, the males may recover, as they are not killed, in contrast to the attract and kill technique, where the pheromones are coupled with insecticides. The four mating disruptor products registered for use against *T. leucotreta* in citrus are: Isomate FCM (Shin-Etsu Chemical Co., Ltd., Japan), Checkmate FCM (Suttera, Bend, OR USA); Splat FCM (ISCA Technologies, Riverside, CA, USA); and X-Mate FCM (Insect Science (Pty) Ltd., Tzaneen, SA) (Moore, 2021). This technique should also be initiated during the early season when the *T. leucotreta* population is low (de Jager, 2013; Malan et al., 2018; Moore, 2022).

Sterile insect technique (SIT) involves release of large numbers of sterile insects into the target areas to induce sterility into the wild population (Mounika et al., 2022). This is the main topic of the thesis and will be discussed in detail (see “1.6 sterile insect technique” below).

1.5.5.5 Biological control

Biological control involves the introduction and utilisation of specific natural enemies (parasitoids and predators) and pathogens (viruses, bacteria, fungi, and nematodes) to successfully control certain pests (Murdoch et al., 1985; Malan et al., 2018; Moore, 2021).

Several options have been tried to control *T. leucotreta* in South African citrus orchards, including parasitoids, entomopathogens, sterile insect technique, and predators (Carpenter et al., 2007; Gendall, 2007; Malan et al., 2018; Moore, 2021). For a biocontrol agent to be effective, it must be host-specific, have a similar life cycle duration to that of the pest, be self-replicating and self-regulatory, and have a high capability of locating the pest (Murdoch et al., 1985).

1.5.5.5.1 Parasitoids

The most effective biocontrol agent against *T. leucotreta* is the egg parasitoid, *Trichogrammatoidea cryptophlebiae* (Nagaraja) (Hymenoptera: Trichogrammatidae) (Willers et al., 1981). Where uninterrupted, parasitism by naturally occurring *T. cryptophlebiae* reached 80-100%, resulting in a reduction in *T. leucotreta* infestation by 67% in Navel oranges, between December and harvest time, resulting in complete suppression of noticeable infestation by harvest (Moore & Hattingh, 2012; Moore, 2021). However, the effectiveness of *T. cryptophlebiae* in the control of *T. leucotreta* can be variable due to several abiotic factors (Catling & Aschenborn, 1974). In addition, *T. cryptophlebiae* is highly sensitive to chemical pesticides, and the choice of inappropriate treatments against other pests may unintentionally kill the parasitoids, reducing them to ineffective levels (Moore & Hattingh, 2012; Moore, 2021).

Augmentation releases of *T. cryptophlebiae* to determine its effectiveness were conducted in the 1970s and 1980s (Schwartz, 1977; Newton, 1988). *Thaumatotibia leucotreta* larval infestation of fruit was reduced by 75% as a result of releases of more than 862,000 parasitoids per hectare over 3 months (Schwartz, 1980). The release of a total of 1.5-3.8 million parasitoids per hectare, divided between weekly releases, reduced the *T. leucotreta* larval population by 61%, although the results were variable (Newton & Odendaal, 1990). This strategy was referred to as inundative release by Newton (1988) and Newton and Odendaal (1990). However, the grandiose number of parasitoids and regular releases, as shown in both studies, are likely to be unviable and expensive if carried out on a commercial scale. Recently, a more moderate inoculative-release approach has been shown to be more effective (Moore, 2022). *Thaumatotibia leucotreta* infestation has been reduced by up to 60% with the release of as few as 100,000 parasitoids per hectare per season (Moore, 2021; Moore, 2022). The initiation of early releases is crucial in achieving this level of success. Releases initiated in October (spring) resulted in a greater reduction in *T. leucotreta* infestation than those initiated during November and December (Moore & Hattingh, 2004).

However, in South Africa, the parasitoid is mass-reared by two facilities, Vital Bugs in Limpopo Province and River Bioscience in the Eastern Cape Province, for augmentative releases in citrus orchards and other crops (Malan et al., 2018; Moore, 2021).

Additionally, parasitoids have also been integrated with other control strategies such as SIT to improve on their effectiveness. Carpenter et al. (2004) demonstrated that use of both egg parasitoids and SIT improved on the control of *T. leucotreta*. Similarly, Bloem at al. (1998) demonstrated that use of sterile *C. pomonella* and *Trichogramma platneri* Nagarkatti (Hymenoptera: Trichogrammatidae) in field-cages had an additive suppressive effect than using either method alone. This observation corresponds with studies by Nagy (1973), Cossentine et al. (1996) and Botto & Glaz (2010), which found that combining the release of sterile insects with *Trichogramma* spp. egg parasitoids achieved a suppressive effect on *C. pomonella*.

Several other species of parasitoids that occur naturally in South Africa are known to attack *T. leucotreta* larvae, aiding in pest suppression. These include the braconids *Apanteles* sp. and *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae) and the ichneumonid *Apophua leucotreta* (Wilkinson) (Hymenoptera: Ichneumonidae), which have been reported to parasitise *T. leucotreta* (Adom et al., 2021). The most effective among these larval parasitoids is *A. bishopi* which has been reported to parasitise up to 40% of 1st and 2nd instar *T. leucotreta* in the Eastern Cape (Gendall, 2007; Zimba et al., 2016a, 2016b). Despite this, no mass-rearing or augmentation programme exists for this or any of the other species. As a result, the only advantage obtained from them is through conservation by following a biointensive IPM approach to avoid killing the naturally occurring population by pesticide use (Moore, 2021). The effectiveness of the parasitoids is dependent on the chemicals used since the use of some chemical sprays has a detrimental effect on parasitoids (Moore & Hattingh, 2016; Malan et al., 2018; Moore, 2022).

1.5.5.5.2 Predators

In citrus trees, ants are regarded as important pests. The two most problematic ant species are the endemic pugnacious ant species, *Anoplolepis custodiens* (Smith) (Hymenoptera: Formicidae) and the exotic brown house ant species, *Pheidole megacephala* (Fabricius) (Hymenoptera: Formicidae) (Samways et al., 1982, 1998). They do not directly damage citrus plants but protect honeydew-producing pests from their natural enemies and even move their immature stages to new, better foliage (Samways et al., 1982; Grout & Moore, 2015). These pests include soft-scale insects (*Coccus hesperidum* L., *C. viridis*

(Green), and *Pulvinaria aethiopica* (De Lotto)), and waxy scales (*Ceroplastes brevicauda* Hall and *Ce. destructor* Newstead (Hemiptera: Coccidae); and citrus mealybug, *P. citri* (Risso) (Hemiptera: Pseudococcidae) (Samways et al., 1982; Grout & Moore, 2015). This shows the need to control or eliminate ants in citrus orchards. However, ants are also very effective predators of other insects in citrus orchards, including arboreal pests that pupate in the soil. Both these ant species preyed on larvae of bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), *T. leucotreta,* and *C. capitata* when the larvae dropped from the tree to pupate in the soil in South Africa (Samways et al., 1982; Bownes et al., 2014). Therefore, the banding of the citrus tree trunks with a sticky exclusion barrier (as opposed to poisoning ants) is strongly recommended to citrus growers (Samways et al., 1998; Bownes et al., 2014). The other predators of *T. leucotreta* recorded include *Orius* bugs (Hemiptera: Heteroptera: Anthocoridae) and assassin bugs (Hemiptera: Heteroptera: Reduviidae), which have been reported to prey on *T. leucotreta* eggs and larvae, respectively (Nyiira, 1970). Similarly, shrews have been reported as predators of *T. leucotreta* pupae in the soil (Omer-Cooper, 1939).

1.5.5.5.3 Microbial control agents

The other agents used in *T. leucotreta* biocontrol are microbial control agents (MCA), which include viruses, fungi, and nematodes (Lacey, 2017). In South Africa, *T. leucotreta* has been controlled by different types of MCA. Most viruses used to control insects belong to the family Baculoviridae; these include granuloviruses (GV) (Betabaculovirus) and nucleopolyhedroviruses (NPV) (Alphabaculovirus) (Moore & Jukes, 2023). Cryptogran® (River Bioscience, South Africa) is an entomopathogenic virus-based product that is available in South Africa for the control of *T. leucotreta* (Moore & Hattingh, 2016; Malan et al., 2018; Moore, 2022; Moore & Jukes, 2023); it is made with Cryptophlebia leucotreta granulovirus (CrleGV-SA), an endemic pathogen found in South Africa. For over 20 years, the pathogen has been used in South Africa and has been recorded to cause up to 92% reduction in *T. leucotreta* infestations (Moore et al., 2015; Malan et al., 2018). Cryptex (Andermatt Biocontrol, Switzerland) and Gratham (Chempac (Pty) Ltd, SA) are also products based on CrleGV that are used to control *T. leucotreta* in South Africa (Moore et al., 2015; Moore & Jukes, 2023).

Three entomopathogenic fungi (EPFs) with high pesticidal potential have been identified in a laboratory bioassay against fifth-instar larvae of *T. leucotreta* (Coombes, 2012, 2015; Prinsloo, 2021). These include, two *Metarhizium anisopliae* (Metchnikoff) Sorokin

(Hypocreales: Clavicipitaceae) isolates and one *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) isolate (Goble et al., 2011; Coombes et al., 2015; Rossouw et al., 2023). The three isolates have been identified in the soil obtained from citrus orchards and areas surrounding citrus orchards in the Eastern Cape, South Africa (Goble et al., 2010; Coombes, 2012; Prinsloo, 2021). Commercial production of the three isolates for control against soil-dwelling stages of *T. leucotreta* is in progress. However, two isolates of *B. bassiana* are registered for the control of *T. leucotreta* on citrus. These are foliar sprays that target the eggs and neonate larvae. However, their mode of application is not satisfactory due to the ultraviolet sensitivity of the fungi, which shortens their residual activity and limits their efficacy (Acheampong et al., 2020; Rossouw et al., 2023). In the meantime, the efficacy can be optimised through accurate timing of applications with peaks in *T. leucotreta* egg-laying, and frequent reapplication (Acheampong et al., 2020).

Several entomopathogenic nematodes (EPNs) were found to be virulent to *T. leucotreta* larvae, as demonstrated in laboratory bioassays using inoculations as low as 5 to 20 infective juveniles (IJs)/cm^2 (Malan et al., 2011; Steyn et al., 2017) and on large scale field trials (Moore et al., 2024). The results indicated that the EPNs have the potential to control *T. leucotreta* in the field. These EPNs were *Heterorhabditis* sp., *Heterorhabditis bacteriophora*, *H. baujardi, H. zealandica*, *Steinernema* sp*., Steinernema khoisanae*, *S. litchi* and *S. yirgalemense* (Malan et al., 2011; Steyn et al., 2017). These positive results have led to the development of Cryptonem® (River Bioscience, South Africa), a product based on the EPN, *Heterorhabditis bacteriophora* (Heterorhabditidae: Rhabditida), using inert clay as the carrier. It has not been on the market for long and has been registered for use against *T. leucotreta* soil-dwelling life stages between harvest and fruit set the following season. However, it is not species-specific to *T. leucotreta*, so can also be used to control other soil-dwelling pests (Malan et al., 2018).

1.5.5.6 Post-harvest control

There is a wide range of post-harvest phytosanitary treatments used for the disinfection of citrus from key pests, such as *T. leucotreta* and the fruit flies, including Medfly, Natal fruit fly, and oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), that can potentially infest citrus fruit, hence posing a phytosanitary threat to export markets (Grout & Moore, 2015). Treatments include irradiation, heat treatments, fumigation, cold treatments, or a combination of these methods. The most effective post-harvest control method for *T. leucotreta* is cold treatment. This is a phytosanitary risk-

reduction treatment (Malan et al., 2018). Hitherto, the only postharvest disinfestation treatments used commercially against *T. leucotreta* and fruit flies on South African citrus were stand-alone cold treatments and partial cold treatments as a component in a multi-tiered systems approach. Recent regulation measures by phytosanitary markets require that fruit should be exposed to sub-zero temperatures for 22-24 days to ensure probit 9 mortality of any *T. leucotreta* present within exported fruit (Moore & Manrakhan, 2022). This was through studies by Myburgh (1963), with ~23000 larvae, and Myburgh (1965), where ~20000 larvae were used. Similarly, Hofmeyr and Hofmeyr (2005) confirmed a less stringent cold treatment protocol, where no survivor was recorded out of 10442 treated fifth instar *T. leucotreta* in fruit after their exposure to −0.6°C for 16 days. These combined trials verified 99.993% mortality at the 95% confidence level (Finney, 1947; Hofmeyr & Hofmeyr, 2005; Schortemeyer et al., 2011).

1.6 Sterile insect technique

Sterile insect technique is an area-wide integrated pest management (AW-IPM) strategy used to control pests and vectors of diseases (Klassen, 2005; Dunn & Follett, 2017; Enkerlin & Pereira, 2022; Mounika et al., 2022). Being a component of AW-IPM, SIT provides considerable potential and has been used with great success against key agricultural pests to establish pest-free areas (eradication), areas of low prevalence (suppression), or to maintain areas free of pests through prevention or containment (Vreysen et al., 2006; Mounika et al., 2022). SIT is considered a form of behavioural control, using gamma radiation to sterilise mass-reared insects (Knipling, 1955; Dunn & Follett, 2017; Mounika et al., 2022). The released sterile pest insects must outcompete the wild male pests in the target sites sufficiently to achieve sterility in the wild population by overcoming the intrinsic rate of increase of wild females (Bartlett & Staten, 1996; Lance & McInnis, 2005; Barnes et al., 2015; Hofmeyr et al., 2015; Gaspar, 2016; Pérez-Staples et al., 2021; Huisamen et al., 2022; Mounika et al., 2022). This strategy achieves control once it is done repeatedly over multiple generations.

1.6.1 History of SIT

Research on how to produce sterile insects started a long time ago. In the 1930s and 1940s, this idea was independently formulated by Edward Knipling, working at the United States Department of Agriculture (USDA), A. S. Serebrovskii affiliated with Moscow State University, and F. L. Vanderplank stationed at a field research facility in Tanzania (Klassen,

2005; Arthur et al., 2015; Daniel, 2016; Mounika et al., 2022). The first SIT programme was started by Edward Knipling and Raymond Bushland in the USA. This was to control New World screwworm, *Cochliomyia hominovorax* (Coquerel) (Diptera: Calliphoridae) (Klassen & Curtis, 2005), a deadly livestock and warm-blooded animal parasite. Herman Muller played a great role in the development of the SIT control programme; in 1920, he discovered the ability to induce sterility in the vinegar fly, *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) using a dentist's X-ray machine (Mounika et al., 2022). Bushland and Hopkins (1953) researched this further and discovered that screwworm males were more susceptible to radiation treatment than females.

The first release attempt of irradiated screwworm pupae was conducted on Sanibel Island, near Tampa, Florida, USA in the late 1940s (Bushland & Hopkins, 1953; Bartlett & Staten, 1996). After four decades of SIT, the technique succeeded in eradicating screwworms in the USA, Mexico, and Panama (Bartlett & Staten, 1996; Klassen, 2005). Serebrovskii (1940) conducted some studies in the 1930s and 1940s focusing on *D. melanogaster*. The latter author aimed to improve agriculture in the Soviet Union by use of chromosomal translocations, that cause the production of non-viable offspring, aiding the eradication of the pest (Serebrovskii, 1940). This study established the extent to which sterility would occur as an inherited trait, supporting the principles of Mendelian genetics. In another study conducted by Vanderplank (1948) at a Tanzanian tsetse fly field research station, he discovered a method for inducing sterility by crossbreeding two fly species, *Glossina swynnertoni* (Austen) and *G. morsitans* (Westwood) (Diptera: Glossinidae), producing sterile hybrids. This discovery helped in the eradication of *G. swynnertoni* in Tanzania (Vanderplank, 1948).

1.6.2 Biological aspects of SIT

The success of SIT application is dependent on (1) the ability to mass-rear, sterilise, and release a sufficient number of sterile males that will achieve an overflooding (sterile to the fertile insect) ratio in the field; (2) the ability of the sterilised insects to maintain their mating competitiveness in the field; and (3) no immigration of fertile insects into the target population from outside the targeted release area (Mounika et al., 2022). Even though the SIT concept seems simple, its implementation is sophisticated (Seawright, 1988). Numerous important aspects should be taken into consideration when deciding on the implementation of SIT to control a given pest in a target geographical area (Lance & McInnis, 2005). These biological aspects eventually determine the logistical practicability and economics of controlling a target insect population using SIT, although political and economic

considerations may affect decisions on where and when the control technique is developed and used (Lance & McInnis, 2005).

Understanding crucial aspects such as pest biology, the role of the pest in the agro-ecosystems, pest ecology and population dynamics, mating systems, post-copulatory factors, and the ability to enhance sterile insect competitiveness will eventually determine if SIT will be effective in the control of an insect population (Lance & McInnis, 2005). The purpose of SIT is to cause a reduction in the population of the target species. Some examples of successful programmes include those with tsetse fly, *Glossina* spp., which transmits trypanosomes to humans and animals, the causative agent of trypanosomiasis (Feldmann et al., 2005), *T. leucotreta* (Hofmeyr et al., 2015; Moore, 2021), and Medflies in Los Angeles in California, USA, preventing the establishment of incursions from Mexico (Dowell et al., 2000; Pérez-Staples et al., 2021). The feasibility of SIT is high when used to control low levels of pests attacking important crops or people (Parker & Mehta, 2007). Ideally, the success of SIT is dependent on changes in the target insect population and the overfloodingg ratios of the irradiated to wild male insects, which need to be continuously maintained to achieve suppression of the pest population (Parker & Mehta, 2007). Overflooding ratios is a key component in the success of SIT programmes. Several SIT studies have been conducted to determine the overflooding ratios of different insect species. Hight et al. (2005) determined that a release ratio of 5:1 in *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) was sufficient to introduce sterility in wild population. Similarly, studies by Villaseñor et al. (2000) indicated that an 80:1 ratio was the lowest overflooding ratio required for controlling *C. capitata*, while Steiner (1969) suggested a 20:1 ratio as crucial for eradicating populations of *Bactrocera dorsalis*, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae), and *C. capitata.*The interaction of sterile insects with wild insects exhibit inverse density dependence. The increase in the number of sterile matings causes a decline in the wild population, eventually suppressing or even eliminating the pest (Enkerlin & Pereira, 2022). However, the latter is a rarity. This makes it possible to reduce pest population levels as well as export the protected commodity to areas where the pest has quarantine restrictions (Parker & Mehta, 2007; Enkerlin & Pereira, 2022). Some biological features such as sexual reproduction and low levels of intrinsic increase are also crucial in the feasibility of a SIT programme, while natural parthenogenesis, aggregated mating systems in eusocial insects, and a long-life cycle in some insects could invalidate the use of SIT (Lance & McInnis, 2005). The receptiveness of a female and the quantity or quality of male gametes produced by a male can also affect the matings between sterile males and wild females (Parker & Mehta,

2007). Regularly monitoring the mating dynamics and compatibility between untreated and treated insects is essential. This is because there is a possibility that genetic changes occurring in laboratory-reared colonies can lead to altercations in reproductive mechanisms viz, pheromone emission, detection, and mating behavior as suggested by Whitten & Mahon (2005). For instance, research conducted by Hiblno & Iwahashi (1991) demonstrated that female melon flies *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) on Okinawa Island were less inclined to mate with treated males compared to wild males. Similarly, Weldon (2005) discovered that mass-reared male Queensland fruit flies *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) modified their calling and mating behavior due to unnatural selection pressures in a laboratory environment focussed on mass-rearing. It's worth noting that resistance to treated insects by wild individuals is exceedingly rare and has not been documented in any significant SIT programme (Whitten & Mahon, 2005).Seasonality and environmental factors, such as temperature, humidity, and photoperiod, affect the development and behaviour of different target insects. Temperature affects the rate of reproduction, longevity, and survival and could also affect the competitiveness of sterile insects with wild females in the field (Nepgen et al., 2015). Due to these effects, wild *T. leucotreta* are active at lower temperatures (below 15°C) than laboratory-reared *T. leucotreta*, which greatly affects the SIT programme (Nepgen et al., 2015). The dispersal and dispersion ability of insects also influences the efficacy of a SIT programme. A target population with an evenly scattered distribution over a large area is conducive to a more effective SIT programme. The pest dispersal capability is key in the use of SIT in AW-IPM programmes, as it is crucial to isolate the treatment areas to curb reinvasion (Klassen, 2005). Insects' chemical communication capabilities have crucial implications for SIT programme. Male insects detect semiochemicals generated by females to recognise and locate a suitable potential mate. In some species, synthetic sexual attractants (pheromones) can be used to optimise mating competitiveness, increasing the efficacy of the SIT programme (Pereira et al., 2013). Synthetic pheromones are also used to evaluate the distribution and over-flooding ratios of released sterile and wild insects (Lance & McInnis, 2005; Nepgen et al., 2015).

Insects with complete metamorphosis make SIT simpler than for direct metabolous insects. This is due to the presence of a quiescent pupal stage that facilitates mass-rearing, harvesting, inducing sterility, and movement of mass-reared sterile insects (Lance & McInnis, 2005). Diapause and length of the insect life cycle should be considered in the development of suitable rearing facilities. Insect phenotype and its ability to compete with wild type are affected by environmental variation in the field and in the rearing facility. The quality of

sterile insects produced is also affected by the artificial diet provided, insect handling methods, and the levels of irradiation used on the insects (Nepgen et al., 2015).

1.6.3 SIT radiation technology

Ionising radiation has been used for a plethora of applications in agriculture, industries, and medicine (Arthur et al., 2015; International Atomic Energy Agency (IAEA), 2018; Mounika et al., 2022). Currently, the common methods used to render insects reproductively sterile for the AW-IPM programmes that include SIT, are gamma radiation, X-rays and gene drive (Bakri et al., 2021). However, chemosterilisation has also been used to induce sterility but has not been operationalised due to the risks of its carcinogenic, teratogenic, and mutagenic side effects on the environment and human health (Baxter, 2016). The main effect of ionising radiation is the breakdown of molecules in the irradiated material or organism. In organisms, mitotically active cells (stem and germ cells) are the most radiosensitive cells (Bakri et al., 2021). In SIT, ionising radiation makes the insect reproductively sterile by destroying the gonial cell's chromosomes (Bloem et al., 2009). This results in germ-cell chromosomal fragmentation, resulting in dominant lethal mutations, chromosomal aberrations, and translocations, where unbalanced gametes are produced, which result in mitosis inhibition and eventually death of fertilised eggs or embryos (Bakri et al., 2021).

Irradiation of insects to induce sterility is a simple, straightforward process using an efficient control procedure. The dosage (radiation absorbed dose) is of prime importance in inducing sterility in the insects. It is expressed in SI units as Gray (Gy) in which 1 Gy = 100 rad and is equivalent to 1 joule (J) of absorbed energy in 1 kg of a specified material (1 Gy = 1 J/kg) (Bakri et al., 2005, 2021). The dose applied to the insects is important, as when the dose is too low, they become partially sterile, while those that receive too high a dose seem to be uncompetitive; the latter results in reduced effectiveness of the SIT programme, requiring a very high number of sterilised insects to be released (Parker & Mehta, 2007).

Different properties affect the suitability of the radiation method used in SIT. They include relative biological effectiveness (RBE), penetrability, availability, cost, and safety. The RBE of radiation is defined as a relative measure of the damage done by a given type of radiation per unit of energy deposited in biological tissues. Gamma radiation, high-energy electron beams, and X-rays all have the same RBE in insects and are considered suitable radiation types to be used in SIT programmes globally (Bakri et al., 2005; Arthur et al., 2015). The appropriate development stage used during the preparation for irradiation is

determined by the maturity of the insect reproductive system, handling procedure during irradiation and transport, as well as sensitivity to somatic change. Germ tissues in most holometabolous insect species are already formed by the last pupal stage or in the early adult stage, making these appropriate stages for irradiation (Bakri et al., 2005). The use of the appropriate radiation dose on each insect species ultimately results in the formation of sterile insects that remain sexually competitive in the field (Arthur et al., 2015).

1.6.4 Successful SIT programmes worldwide

One of the successful SIT programmes in the world was the control of New World screwworms in the USA by Edward Knipling and Raymond Bushland in the 1950s, which led to the total eradication of the pest (Baumhover et al., 1955). During the 1920s, Herman Muller played a role in the creation of the SIT programme through his discovery of inducing sterility in the vinegar fly using a dentist's X-ray machine (Muller, 1928). The method of using radiation treatment to produce sterility in insects was further studied by Bushland and Hopkins (1953), who discovered that males were more susceptible to the treatment than females. This led to the first small-scale radiation and release of screwworm pupae on Sanibel Island, Florida in the 1940s by Bushland and Hopkins (1953). However, several other insect species have been successfully suppressed and occasionally even eradicated using SIT programmes (Bartlett & Staten, 1996).

Similarly, another successful and best-known SIT programme was the elimination of tsetse flies (*Glossina austeni* Newstead) in Zanzibar between 1994 and 1997 (Vreysen et al., 2000). Tsetse flies were major vectors of trypanosomiasis on the Island of Zanzibar, causing nagana in domestic animals and sleeping sickness in humans. The disease led to estimated annual losses of about 2 million USD in terms of milk and meat production, death of calves, and the high cost of disease control (Vreysen et al., 2014). The trials to control tsetse fly began in 1908 when they were first reported in Zanzibar, using several methods such as bush clearing, the extermination of wild animal hosts, spraying of insecticides on the hosts, the fly habitat, and the use of traps. These techniques reduced tsetse populations; however, they did not provide long-lasting control of the vector (Vreysen et al., 2014). In 1994, the SIT programme was integrated with other control strategies in Zanzibar as an environmentally benign technique. This led to the total eradication of tsetse flies, providing a long-lasting solution (Msanga et al., 2000; Vreysen et al., 2014).

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), was successfully controlled in the San Joaquin Valley, California, USA in 1968

using SIT (Bartlett & Staten, 1996; National Cotton Council of America (NCCA), 2001; Daniel, 2016: Marec & Vreysen, 2019). The sterile PBW insects were continuously released weekly during the cotton growing seasons from 1968 to 1992. The California growers and the state contributed finance annually to help in the production of sterile PBW adults at a rearing facility in Phoenix, Arizona. From 1968 to 1991, the PBW mass-rearing facility in Phoenix produced 99-826 million dye-marked PBW (Bartlett & Staten, 1996). The sterilised PBW were released from aircraft in cotton-growing parts of San Joaquin Valley. This programme, along with other integrated control strategies, such as the use of Bt cotton and the application of pheromones for mating disruption, led to the successful suppression of the PBW in the areas around southern California (including San Joaquin Valley), Arizona, and Mexico (Bartlett & Staten, 1996; NCCA, 2001; Daniel, 2016; Marec & Vreysen, 2019).

Medfly has also been successfully controlled and eliminated in some countries using SIT programmes. The SIT programme against Medfly was started in southern Mexico in 1977; this was to prevent the further establishment of Medfly in Central America, Mexico, and the USA. A rearing facility that produced a total of 500 million sterile medflies was established in Tapachula (Hendrichs et al., 2002). In 1982, Medflies were successfully eradicated in southern Mexico. Since then, a sterile fly barrier was established between southern Belize and Guatemala to southern Mexico (Villaseñor et al., 2000); this has led to the maintenance of a sterile fly barrier in the area, enhancing a Medfly-free status in Mexico and the USA (Hendrichs et al., 2002). This technique has also been used in Argentina, Chile, Dominican Republic, and Peru helping in the eradication of the pest (Plá et al., 2021). In Chile, the conventional use of insecticides did not succeed in eradicating the Medfly (Olalquiaga & Lobos, 1993); however, eradication of the pest in Chile was achieved in 1995 with the integration of SIT. This enabled more trade opportunities for over 5 years, benefitting the Chilean fruit industry by ~500 million USD (Servicio Agrícola y Ganadero (SAG), 1996).

In Argentina, SIT programmes started in the early 1990s against Medfly managed to develop Medfly-free areas in various Patagonian valleys. As a result, Argentina succeeded in negotiations with Chile to transport fruit from Mendoza and Patagonian regions through Medfly-free Chile for export from Chilean ports (de Longo et al., 2000). In Valencia, Spain, Medfly was a serious problem, threatening the cultivation of citrus and other fruits. For over 50 years, insecticides were used in the area, but this did not achieve long-term control. This led to the implementation of a SIT programme to control fruit flies in 2007, resulting in the suppression of fruit flies there, reducing losses and increasing exports from the area (Plá et

al., 2021). SIT programmes have been implemented globally to manage various insect species, but not all of these programmes have been successful. Some programmes are still ongoing, while others have been terminated due to their lack of effectiveness (Table 1.6).

Table 1.6: Several examples of insect pest species for which SIT has been used, is currently being used or is being developed (Brown & Aliniazee, 1978; Seligman et al., 1990; Villaseñor et al., 2000; de Longo et al., 2000; Klassen & Curtis, 2005; Orankano et al., 2007; Vreysen et al., 2010; Oliva et al., 2012; Barnes et al., 2015; Hofmeyr et al., 2015; Plá et al., 2021; Mounika et al., 2022; IAEA, 2023).

Target insect	**Species name**	**Location**	**Outcome**	**Release type**
Screwworm	*Cochliomyia hominivorax* (Coquerel)	Curaçao, USA, Mexico, Puerto Rico, US Virgin Islands, Guatemala, Belize, Libya, Costa Rica & Panama	Eradicated	Area-wide releases
Mediterranean fruit fly	*Ceratitis capitata* (Wiedemann)	San Francisco Valley-Brazil, Italy, Peru, Mexico, California-USA Argentina, Australia, Chile, Dominican Republic, Israel, Hex	Eradicated in some areas; Population suppression achieved in other areas	Area-wide releases

		River Valley-South Africa, Valencia-Spain, Morocco, Cap Bon-Tunisia, Morocco & Mauritius		
Melon fly	*Zeugodacus curcubitae* (Coquillett)	Okinawa-Japan & Mauritius	Eradicated in Japan; Reduced infestation and damage to fruit in Mauritius	Area-wide releases
Peach fly	*Bactrocera zonata* (Saunders)	Mauritius	Reduced infestation and damage to fruit	Pilot release
Oriental fruit fly	*Bactrocera dorsalis* (Hendel)	Rota, Hawaii, Mauritius & Ratchaburi Province-Thailand	Reduced infestation and damage to fruit	Area-wide release
Onion fly	*Delia antiqua* (Meigen)	Holland	Not feasible	Area-wide releases
Mexican fruit fly	*Anastrepha ludens* (Loew)	USA & Mexico	Started to eradicate then continued as a containment	Area-wide release

			programme	
Cherry fruit fly	*Rhagoletis cingulata* (Loew)	Switzerland	Substantial reduction in cherry infestation rates and subsequent progeny production	Pilot release
Codling moth	*Cydia pomonella* (Linnaeus)	British Columbia-Canada	Eradication was achieved in localised areas, whereas population suppression in other areas of Canada	Area-wide release
Spotted wing drosophila	*Drosophila suzukii* (Matsumura)	Argentina	Partial sterility	Pilot release
Pink bollworm	*Pectinophora gossypiella* (Saunders)	California-USA	Prevented the spread of bollworm to surrounding areas	Area-wide releases
Gypsy moth	*Lymantria dispar* (Linnaeus)	Massachusetts-USA	Unknown	Area-wide release
False codling moth	***Thaumatotibia leucotreta* (Meyrick)**	**Eastern and Western Cape Provinces-South Africa**	**Population suppression achieved**	Area-wide release

Tsetse fly	*Glossina* spp.	Tanzania, Nigeria, Senegal, Slovakia & Uganda	Eradicated in Tanzania; Population suppression in other areas	Area-wide releases
Boll weevil	*Anthonomus grandis* (Boheman)	Southern Mississippi-USA	Population suppression achieved	Area-wide releases
Western Encephalitis mosquito	*Culex tarsalis* (Coquillett)	USA	No population suppressed	Small field tests
Asian tiger mosquito	*Aedes albopictus* (Skuse)	Reunion Island, Mexico, China, Italy	Two-fold reduction of wild population's fertility	Pilot releases
Sweet potato weevil	*Cylas formicarius* (Fabricius)	Kume Island-Japan	Eradicated	Area-wide release
New World malaria mosquito	*Anopheles albimanus* (Wiedemann)	Lake Apastapeque-El Salvador	97% population reduction	Area-wide release
Cockchafer	*Melolontha hippocastani* (Fabricius)	Switzerland	100% reduction of target population	Field releases
Yellow fever mosquito	*Aedea aegypti* (Linnaeus)	Cuba, Malaysia, Mexico, Indonesia, Singapore, USA	Partial sterility	Field releases
Southern house	*Culex*	New Delhi-	90% sterility	Area-

mosquito	*quinquefasciatus* (Say)	India	of the eggs	wide release
House mosquito	*Culex pipiens* (Linnaeus)	France	Population reduction	Pilot release
Cactus moth	*Cactoblastis cactorum* (Berg)	Yucatán-Mexico	Population eradication	Area-wide release
Sugarcane stalk borer	*Eldana saccharina* (Walker)	KwaZulu-Natal-South Africa	Population reduction	Pilot release

SIT is suitable for the control of certain pests that attack crops, livestock, and humans. It has also been championed because it is environmentally benign and can be integrated with other control strategies, as it is species-specific; also, chemical insecticide usage can be avoided or reduced, thereby leaving no residues on the produce or in the environment (Lance & McInnis, 2005; Daniel, 2016).

1.6.5 *Thaumatotibia leucotreta* SIT programme in South Africa

Despite *T. leucotreta* being endemic to the sub-Saharan region, it was only recorded in the Western Cape Province, South Africa in 1969 (J.G. Louw, per. Comm. in Hofmeyr et al., 2015) as infestation in pears on a fruit farm in the Paarl region. The pest later spread and in 1976, it was recorded in the Olifants River Valley, ~180 km North of Paarl, on a Navel orange farm adjacent to a hot-water spring holiday resort, where it was believed that infested oranges with *T. leucotreta* might have been discarded by visiting tourists (A.J. Marais, pers. Comm. in Hofmeyr et al., 2015). *Thaumatotibia leucotreta* is now established in the western and southern parts of the Western Cape Province (Hofmeyr et al., 2015; Boersma, 2021). Several measures have been used to control the pest, such as insecticides, orchard sanitation, and mating disruption. However, the use of chemical insecticides was not sustainable. The presence of *T. leucotreta* represented a high phytosanitary threat to South African export markets. The situation was worsened when the *T. leucotreta* developed resistance against certain registered pesticides and stringent regulations were imposed on exporters (Hofmeyr & Pringle, 1998). This resulted in a zero tolerance for *T. leucotreta* and the prerequisite for post-

harvest cold treatment, initially by the USA in 1999 (Hofmeyr & Hofmeyr, 2005; Hofmeyr et al., 2016).

Due to pesticide resistance and restrictions on pesticide residues for export fruit, a long-term solution was required. Although some control tactics, such as the use of insecticides, mating disruption, and orchard sanitation, had been used to some extent to manage the pest, a more environmentally friendly control tactic was needed. Similarly, in their preliminary studies, Myburgh (1963), Schwartz (1979), and Du Toit (1981) explored the effects of gamma radiation on *T. leucotreta* in South Africa. However, these studies did not address the issue of partial sterility, which results in more competitive *T. leucotreta* used in SIT programmes (Carpenter et al., 2007). They discovered that significantly lower doses of gamma radiation were necessary to induce sterility than those required for mortality. The ova were almost entirely sterilised at a dose of around 30 kiloroentgen (Kr), whereas the males were less sensitive. Sperm, on the other hand, was sterilised at a dose between 60 and 70 Kr (Myburgh, 1963). However, Myburgh (1963) also considered the use of gamma-irradiated sterile insects for pest control but concluded that this method was impractical due to the widespread distribution of the pest and the large number of sterile insects that needed to be released. Therefore, in 2002, research began to develop an AW-IPM programme with a SIT component for *T. leucotreta* (Klassen & Vreysen, 2021). CRI, CGA, Joint Food and Agriculture Organization of the United Nations/International Atomic Energy Agency (FAO/IAEA), USDA through Agriculture Research Council (ARC) and the Centre for Plant Health Science and Technology (CPHST), collaboratively joined resources and efforts to develop and test the effectiveness of SIT programme for *T. leucotreta* (Hofmeyr et al., 2015; Gaspar, 2016; Boersma, 2021).

1.6.5.1 Developmental phases of SIT control against *T. leucotreta*

Research on the SIT control project against *T. leucotreta* was conducted from 2002 to 2007 in Citrusdal, Western Cape Province in South Africa in five phases (Hart, 2012; Marec & Vreysen, 2019):

Phase 1 (2002-03): Radiation biology and inherited sterility studies

In *T. leucotreta*, a study to determine the suitable radiation dosage was conducted by Bloem et al. (2003). This was the first phase in the development and commercialisation of SIT in *T. leucotreta*, conducted in Citrusdal, Western Cape Province in South Africa. Eight doses of ionising radiation, ranging from 0 to 350 Gy in 50 Gy increments, were used to treat

T. leucotreta pupae and moths. Different couplings of sterile (S) and fertile (F) moths were inbred (S♀ × S♂; F♀ × F♂) and outcrossed (S♀ × F♂; F♀ × S♂). Results on fecundity and fertility indicated 100% sterility was achieved by a dose of 200 Gy when *T. leucotreta* were irradiated in pupal and adult stages in crosses involving (F♂ × S♀) (Bloem et al., 2003).

Phase 2 (2005): Field cage study

The second phase of the project followed the promising results of the first phase. This is discussed in detail in Chapter 2, due to the relatedness with our study.

Phase 3 (2005-06): SIT pilot study

The SIT pilot project started at the end of 2005 in commercial citrus orchards in Citrusdal (Hofmeyr et al., 2015). This was carried out in 35 ha of mature Navel orange orchard. The citrus orchard was surrounded by natural vegetation without any known alternative *T. leucotreta* host plants. The nearest untreated citrus orchard was 600 m away. Five ha of untreated Navel orange orchard, situated 800 m away from the SIT site, was used as a control. Orchard sanitation was conducted on both experimental farms once a week to dispose of all the dropped and damaged fruit. The moths used in the experiment were 24 h old individuals treated with 150 Gy. Releases were carried out for 29 weeks from November 2005 until the harvest period in June 2006, with a total of 1000 sterile moths released twice a week. Different colours of fluorescent powder (DayGlo Color Corp., Cleveland, Ohio, U.S.A.) were used to topically mark each successive batch of released moths to aid in the recognition of sterile and wild moths in the traps using UV light. Thirteen delta traps were used, evenly spaced over the orchards, each equipped with synthetic pheromone (Cardiff Chemicals, Cardiff, UK) in Lorelei dispensers (CRI, Citrusdal) with sticky floors. Two traps were used in the control orchard. The objective was to create an overflooding ratio within the release orchard, of not less than 10 sterile: 1 wild male *T. leucotreta* per week (Hofmeyr et al., 2005; Hofmeyr et al., 2015). The promising results from the pilot project study indicated a mean crop loss to *T. leucotreta* infestation of 0.1 and 2.1 damaged fruit per tree per week in the SIT and control site, respectively, which was a 95.2% infestation reduction using SIT. During the last 6 weeks before harvest, fruit drop due to *T. leucotreta* infestation averaged 0.2 and 6.1 fruit per tree in the SIT and control orchards, respectively, which was a 96.7% reduction in crop loss (Hofmeyr et al., 2015).

Phase 4 (2007): Construction of a mass-rearing facility

The SIT study showed some promising results in phases 1-3, which was convincing evidence to the southern African citrus industry, indicating that the SIT technique should be used as a suppression tool against *T. leucotreta* (Hofmeyr et al., 2015). Consequently, the CGA decided to expedite the adoption of this technique. As a result, a private company, X Sterile Insect Technique (XSIT) (Pty) Ltd., was established in 2006. The company was responsible for the production, sterilisation, and release of sterile *T. leucotreta* in the Citrusdal region. To boost moth production, new equipment was designed to replace the earlier infrastructure developed for small-scale *T. leucotreta* production (Hofmeyr et al., 2015). The new mass-rearing facility was designed to produce 21 million *T. leucotreta* moths per week. In 2007, the upscaled new mass-rearing facility was operationalised, and in November of the same year, the release of irradiated moths commenced (Hofmeyr et al., 2019). Moth production increased from 4 million per week in the 2007-2008 season to about 8 million to 12 million moths per week in the 2008-2009 and 2009-2010 seasons, respectively (Hofmeyr et al., 2015). Currently, the expanded facility produces about 50 million sterile moths per week, servicing more than 19500 hectares of citrus and table grapes in South Africa (Gaspar, 2016; XSIT, 2018).

Phase 5 (2007-2018): Commercial area-wide implementation of sterile insect releases

After the commercialisation of SIT in 2007, XSIT aimed to conduct *T. leucotreta* releases in all citrus orchards (6500 ha) in the Citrusdal region, in a phase-in over four years. About 2000 moths per week were released in the first year (2007-2008) for 8 months (October 2007 to April 2008) in about 1500 ha of citrus orchards (Hofmeyr et al., 2015). To enable reliable sorting of released sterile and wild moths in the traps, Calco Oil Red® (Royce International, Sarasota, Florida, U.S.A.) was added to the *T. leucotreta* rearing diet. By 2009-2010, an additional 4000 ha in Western Cape Province were included in the SIT programme (Hofmeyr et al., 2015). By 2018, SIT was also being conducted in over 6500 and 2200 ha of the Sundays River and Gamtoos River Valleys in Eastern Cape Province, respectively (Hofmeyr et al., 2019). In the 2016-17 season, SIT was expanded to the lower Orange River Valley region in the Northern Cape Province and the Hex River Valley of the Western Cape Province (where the latter is a crucial table-grape producing region), with 1500 and 4000 ha treated, respectively (Boersma, 2021). By 2018, XSIT was supplying sterile *T. leucotreta* moths to more than 19500 ha every week (XSIT, 2018; Boersma, 2021). Consequently, there was an increased seasonal improvement in wild *T. leucotreta* suppression due to regular releases of sterile moths in all the treated regions. As a result, in the Olifants River Valley,

the average number of wild male moths caught in traps dropped from 13.0 per trap per week before sterile moths were released in 2006, to 2.0, 0.4, and 0.1 per trap per week in 2012, 2013, and 2014, respectively (Barnes et al., 2015). Fruit infestation by *T. leucotreta*, based on the inspection of fallen fruit from 3,195 data trees, dropped from 2.6% in 2010/11 to 0.1% in 2013/14 (Barnes et al., 2015). Additionally, there was a reduction in crop losses and fewer rejections of export fruit consignments due to the presence of *T. leucotreta*. According to the Perishable Products Export Control Board in 2013, the percentage of fruit rejections in Western Cape Province dropped from 6.70% of fruit originating from non-SIT orchards to 0.88% from SIT orchards (Barnes et al., 2015; Boersma, 2021). Although the growth of the SIT programme was rapid, it was associated with numerous challenges and hardships that sometimes threatened its effectiveness and existence.

To overcome some of these challenges, and to sustain the target sterile to wild ratio (10:1) in an area, additional numbers of moths sometimes had to be released. This was done by increasing the number of release times per week (Hofmeyr et al., 2019). To curb the deterioration of quality and ensure effective longevity in the field, the moths had to be released within 24 h after irradiation. Transport and distribution of irradiated moths are subject to applied protocols, as moths are transported by road to farms up to 900 km from the rearing facility. Temperatures in the transporting vehicles need to be maintained between 5 and 7°C. In the field, *T. leucotreta* releases are conducted at an ambient temperature between 12 and 28°C (Hofmeyr et al., 2019). Initially, the moths were released using quad bikes and all-terrain vehicles (ATVs). However, in 2010, this was changed to gyrocopters and small fixed-wing aircraft (Nepgen et al., 2015; Hofmeyr et al., 2016, 2019). Currently, releases are done by helicopters, making it possible to release the sterile insects over large geographical areas in a relatively short space of time. The monitoring of aerial releases of the moths is conducted using the Global Positioning System (GPS) at about 30 m above ground level and 130 m flightpath width (Nepgen et al., 2015; Hofmeyr et al., 2019).

1.6.6 Inherited sterility in *T. leucotreta*

Inherited sterility (IS) is otherwise referred to as delayed, induced, partial, or F1 sterility. In SIT programmes, varied levels of sterility can be induced in insects (Mounika et al., 2022). It is suitable for organisms such as hemipterans and lepidopterans that have polycentric chromosomes (Bartlett & Staten, 1996; Mounika et al., 2022). LaChance (1985) determined several attributes that contribute to the effectiveness of IS in some lepidopteran species such as *T. leucotreta* and codling moth. These attributes include radio-sensitivity

differences between the male and female parental generation (P1); more sterile F1 male and female offspring than in the treated P1 generation; production of more male than female progeny in the F1 generation; and prolonged development time and lower sperm quality in the F1 generation (Bloem et al., 2003; Soopaya et al., 2011; Mounika et al., 2022). In this phenomenon, aberrant chromosomes are transmitted from the released sterile population to the wild population (Soopaya et al., 2011). In *T. leucotreta*, the selection of radiation dosage is crucial since the female moths are sterilised with a lower radiation dosage than the male moths. If sterilised at the same dose, partially sterilised *T. leucotreta* males produce F1 progeny with increased levels of sterility (Bloem et al., 2003).

Bloem et al. (2003) determined that *T. leucotreta* males sterilised as pupae and adults with 350 Gy and crossed to fertile females (F♀ × S♂) achieved residual sterility of 3% and 5%, respectively. Bloem et al. (2003) also determined the inherited sterility in the F1 generation by crossing fertile females' eggs to males sterilised with doses that allowed some level of residual sterility (F♀ × S♂). The eggs were incubated in glass jars with a rearing diet, and the resulting F1 progeny (F_1) was inbred and outcrossed with fertile moths (F) (F_1♀ × F_1♂; F_1♀ × F♂; F♀ × F_1♂; F♀ × F♂). Increased F1 mortality during development and reduction in F1 fecundity and fertility were recorded when increasing doses of radiation were applied to the P1 males. Additionally, a significant shift in the F1 sex ratio in favour of males was recorded. When the P1 males were sterilised using a dose of 150 Gy, 100% sterility was achieved in the F1 progeny (Bloem et al., 2003). Therefore, the reproductive competitiveness of the partially sterile *T. leucotreta* male is high compared to that of the fully sterilised females. This phenomenon leads to the production of sterile F1 individuals in the wild population, which aids in suppressing the *T. leucotreta* wild population (Bartlett & Staten, 1996; Nepgen et al., 2015; Mounika et al., 2022).

1.7 Problem statement and justification of the study

SIT is recommended as the primary AW-IPM technology in various regions of South Africa where it is commercially practised. One of the key factors for the successful *T. leucotreta* SIT programme is achieving a sufficient "overflooding ratio" of sterile males to wild males (Klassen, 2005). Sterile males must effectively outcompete wild males in the release area to induce sufficient sterility into the wild population to overcome the intrinsic rate of increase of the pest population (Barnes et al., 2015). The release ratio varies from one insect species to another (Klassen, 2005). For *T. leucotreta*, a minimum ratio of 10:1 sterile males to wild males has been established in a field cage study (Hofmeyr et al., 2005).

Nevertheless, achieving this in a commercial field setting may be more challenging than in a controlled cage environment. Additionally, some field trials suggested that higher release ratios might be more effective (Hofmeyr & Hofmeyr, 2009, 2010; Moore, 2011). Although higher release ratios than 10:1 are usually maintained in the field (XSIT, 2018), this study investigated the effectiveness of these higher release ratios in a laboratory and field cage environment.

Similarly, in the laboratory studies, some crossings of treated (T) male (M): untreated (U) female (F) and TF: UM resulted in some fruit infestation, as reported by Moore et al. (2021). However, van Steenderen (2017) found in both laboratory and field cage mating studies that sterile females are beneficial in the *T. leucotreta* SIT programme, acting as a positive sperm sink (for wild males) rather than a negative sperm sink (for treated males). This finding highlights the need to investigate the impact of different combinations of treated and untreated males and females on fruit infestation and population growth rate in laboratory and field cages.

SIT is not effective as a standalone technique, hence the need to integrate it with other control strategies, especially augmentative biological control, which is environmentally friendly. An effective *T. leucotreta* SIT programme that combines SIT with *T. cryptophlebiae* can only be successful if the two tactics do not negatively affect each other. Carpenter et al. (2004) conducted a laboratory study examining the acceptability and suitability of *T. leucotreta* eggs to parasitism by *T. cryptophlebiae*. This study used only ratios 5:1 and 10:1 of sterile-to-fertile *T. leucotreta*, highlighting the need to investigate the effects of eggs produced from higher release ratios of sterile and fertile *T. leucotreta* on the acceptability and suitability to *T. cryptophlebiae* oviposition and development. The knowledge of the compatibility of *T. cryptophlebiae* and the release of irradiated *T. leucotreta* is crucial to the evaluation of the combined use of these tactics.

The *T. leucotreta* SIT programme involves releasing sterile moths into orchards to mate with the wild population, thereby aiding in pest suppression. However, due to the impracticality and cost of separating males and females, XSIT conducts dual-sex releases (XSIT, 2018). Despite efforts to minimise pre-release matings by storing the insects at 4-6°C to keep them inactive, such matings have been reported (Boersma, 2021). This necessitated the need to investigate the extent of pre-release mating that occurs during the production and release stages of sterile *T. leucotreta*.

1.8 Objectives

1.8.1 Overall objective

The main objective of this study was to enhance the efficiency and effectiveness of the *T. leucotreta* SIT programme by examining the key factors that influence its success.

1.8.2 Specific objectives

a) To compare the efficacy and population growth rate resulting from releases of sterile to fertile *T. leucotreta* at ratios higher than 10:1 (sterile: fertile) with that achieved with the current benchmark of 10:1 in the laboratory and field cage studies.

b) To compare fruit infestation in the laboratory and field cage studies, resulting from different combinations of treated (T) and untreated (U), male (M), and female (F) moths, UM × UF, TM × UF, UM × TF, TM × TF and UM × UF × TM × TF.

c) To investigate the acceptability and suitability of false codling moth eggs resulting from higher release ratios of sterile to fertile moths to parasitism by egg parasitoid *T. cryptophlebiae* in a laboratory cage study.

d) To determine the pre-release mating levels across different stages of production and release of sterile *T. leucotreta* in a SIT programme.

1.8.3 Hypotheses

a) Higher release ratios of sterile to fertile *T. leucotreta* are more efficacious than a release ratio of 10:1.

b) Treated and untreated male and female *T. leucotreta* pairings can suppress fruit infestation and population growth rate.

c) *Thaumatotibia leucotreta* eggs from higher release ratios of sterile to fertile moths are suitable for oviposition by *T. cryptophlebiae*.

d) Sterile male and female *T. leucotreta* pre-release matings occur across the production and release stages in a *T. leucotreta* SIT programme.

1.8.4 Outline of the thesis

This thesis is written as a series of chapters that have been published or are intended for publication. Thus, there is considerable overlap and some repetition, especially in the introductions to the chapters. The abstracts of the chapters have been consolidated at the beginning of the thesis and all of the references have been put into one section to reduce

overlap and the length of the document.

CHAPTER 2

Influence of overflooding ratios on fruit damage and population growth of the *Thaumatotibia leucotreta* (Meyrick): implications for the sterile insect technique programme

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2.1 Introduction

Thaumatotibia leucotreta is an endemic pest to SSA (EFSA, 2024). It is a polyphagous pest that infests a wide array of cultivated and wild fruits and nuts (EFSA, 2024). Amongst the cultivated fruit, citrus is a preferred host, particularly Navel oranges (Economides, 1979; Yahia et al., 2011; Maniania et al., 2017; Moore et al., 2021; Moore & Manrakhan, 2022; EFSA, 2024). In South Africa, *T. leucotreta* has four to six overlapping generations per annum. Females typically lay 100-250 eggs on the fruit rind, and fecundity is influenced by abiotic factors such as temperature, relative humidity, and food quality (Daiber, 1980; Adom et al., 2021; Githae et al., 2024). Upon hatching, the neonates burrow into the fruit, feeding on the pulp, and undergo five larval instars. Late fifth instars exit the fruit to pupate in the topsoil or leaf litter (Moore, 2022). *Thaumatotibia leucotreta* infestation leads to premature fruit drop before harvest, and severe infestations can result in crop losses and potential rejections at packing houses (Hofmeyr et al., 2005; EFSA, 2024). However, advancements in field control measures over the past two decades have reduced the occurrence of severe infestations (Moore et al., 2016, 2017; Moore, 2022).

One of the *T. leucotreta* management options is the use of the SIT (Alphey & Bonsall, 2018). Globally, applied against a range of different pests, this technique has resulted in the production of higher-quality agricultural products, increased crop yields, created job opportunities, and expanded trade routes (Enkerlin, 2005; Klassen & Curtis, 2005). SIT is an autocidal pest control strategy that involves mass-rearing, sterilisation, and release of sterile insect pests to mate with wild fertile insects resulting in pest suppression, eradication, or containment (Carpenter et al., 2009; Alphey & Bonsall, 2018). This technique is regarded as environmentally benign because of its autocidal mode of action, eliminating the need for pesticides, and has led to its widespread adoption in pest control efforts worldwide (Enkerlin, 2005). In South Africa, SIT is currently being practised in the Western Cape, Eastern Cape, and Northern Cape Provinces to control *T. leucotreta*, thereby suppressing it (Hofmeyr et al., 2015; Boersma, 2021). By 2015 the programme in the Western Cape Province was reported to have reduced moth catches by 99%, fruit infestation by 96%, and export rejections by 89% since the inception of the programme in 2007 (Barnes et al., 2015).

The primary goal of SIT is to induce sterility in wild fertile populations as a way of controlling pests (Hendrichs et al., 2002). However, the effectiveness of SIT is dependent on various factors, including the quality of irradiated males, mating competitiveness, and the overflooding ratio (hereafter, OFR) (Alphey & Bonsall, 2018). Mating competitiveness pertains to the ability of released sterile males to successfully compete with wild males to

mate with wild females, resulting in the production of non-viable eggs (Knipling, 1959; Shelly & McInnis, 2016; Zavala-Lopez & Enkerlin, 2016). This is an important aspect to consider, as achieving high numbers of low-quality sterile insects may affect the effectiveness of the technique. The quality and capacity of mass-produced and sterilised males to mate with wild females play a crucial role in determining the success of SIT (Rull et al., 2005; Orozco-Davila et al., 2007). In various lepidopteran species, improvements in the quality of sterile insects have been achieved by reducing the dose of ionising gamma radiation. This has been recorded in the cotton bollworm, *H. armigera* (Osouli & Kalantarian, 2021), the fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) (Carpenter et al., 1996; Rafi et al., 2023), and the codling moth, *C. pomonella* (Fossati et al., 1971; Proverbs et al., 1978; Bloem et al., 1999; Vreysen et al., 2010).

Reduced radiation dose can lead to incomplete sterility in male *T. leucotreta*, otherwise known as inherited or induced sterility (Bloem et al., 2003). Although the released (P1) generation of *T. leucotreta* males are partially sterile, their progeny (The F1 generation) is completely sterile making this approach more effective (LaChance, 1985). Not only is the F1 progeny produced in the field completely sterile but there is sex ratio distortion in favour of males (LaChance, 1985; Carpenter et al., 2009). According to Knipling (1970), to achieve the same level of suppression within a native population over three generations, four times as many fully sterile insects as partially sterile insects would need to be released. This phenomenon in *T. leucotreta* was investigated by Bloem et al. (2003), explored the impact of increasing gamma radiation doses on the reproductive capabilities of *T. leucotreta*, where both pharate adults and mature pupae were subjected to ionising radiations ranging from 0 to 350 Gy. The research revealed that the dose or radiation had a minimal effect on fecundity when non-irradiated females were mated with irradiated males. However, when irradiated females were mated with either non-irradiated or irradiated males, their fecundity significantly declined as the ionising radiation dose increased. Notably, a dose of 200 Gy resulted in 100% sterility in irradiated females. Similarly, the effects of irradiating P1 males with specific dosages were observed in the F1 generation, leading to reduced F1 fecundity and fertility, increased mortality during F1 development, and a significant shift in the F1 sex ratio in favour of males.

Ensuring that the ideal sterile-to-wild ratio is maintained is crucial for enhancing the effectiveness of most SIT programmes. Therefore, considering the findings from Bloem et al. (2003), Hofmeyr et al. (2005) conducted a study to determine the appropriate release ratio for *T. leucotreta*, using walk-in field cage experiments. Newly emerged adult *T. leucotreta*,

subjected to either 150 or 200 Gy gamma radiation, were released into the cages at ratios of five sterile to one fertile individual or 10:1 for four weeks. In control cages, only fertile moths were released. The findings indicated a decline in the number of larval infestations and F1 offspring per cage as the OFR increased. Specifically, the treatment with 150 Gy and a ratio of 10:1 had the lowest average number of fertile F1 adult females and males. Likewise, this treatment also exhibited the lowest rate of increase per generation (<1 from the P1 to F1), suggesting that maintaining *T. leucotreta* releases at this dosage and ratio in the field would suppress the growth of the fertile population. Consequently, an OFR of at least 10 sterile males to one wild male (10:1) was established as the benchmark ratio for effective *T. leucotreta* management (Hofmeyr et al., 2005).

To maintain this ratio in orchards, the number of *T. leucotreta* released may need to be adjusted, depending on wild population density, by either increasing the frequency of releases per week or expanding the width of release zones (Calkins & Parker, 2005). In the *T. leucotreta* SIT programme, a total of 2000 sterile moths are released per week per hectare, but this can be adjusted depending on the season, environmental conditions, and wild population levels (Boersma, 2021). Therefore, having an accurate estimate of the wild population density is imperative to determine the required quantity of sterile insects to be released (Rendón et al., 2004; Orozco et al., 2013; Flores et al., 2014). To accomplish this, the utilisation of synthetic pheromone traps serves as the tool for assessing wild population densities of *T. leucotreta* as well as estimating the sterile-to-wild ratio achieved upon the release of the sterile insects and their distribution in the field (Vreysen, 2005). However, achieving this in a typical field setting might pose challenges that are not encountered in a controlled field cage design. Moreover, certain field trials involving *T. leucotreta* SIT have suggested that employing higher ratios could yield better results (Hofmeyr & Hofmeyr, 2009, 2010; Moore, 2011). While it is true that ratios higher than 10:1 are typically maintained in field applications (XSIT, 2018), it would be prudent to further investigate the effectiveness of these higher ratios. Therefore, this study aimed, through laboratory cage experiments, to investigate whether higher than 10:1 *T. leucotreta* ratios (sterile: fertile) would improve the effectiveness of the technique and reduce the rate of pest population growth, through laboratory cage experiments. The results obtained are discussed in the context of improving the *T. leucotreta* SIT programme to aid in suppressing the pest in South Africa and anywhere else where it may occur.

2.2 Materials and Methods

2.2.1 Test insects

Adult sterile and fertile *T. leucotreta* were sourced from XSIT (Pty) Ltd., Citrusdal, South Africa. To render them sterile, adult moths were exposed to a dose of 180 Gy of gamma irradiation. Thereafter, the moths were transported to Rhodes University, Makhanda, South Africa (~840 km, 13-14 h) in a polystyrene cooler box with dry ice bricks, maintaining a temperature of 4-6°C. This controlled temperature was crucial in minimising the moths' activity and preventing mating while in transit (Nepgen et al., 2015). The moths were approximately 48 h old upon arrival and were kept in a refrigerator at 5°C and immediately sorted by sex using a dissection microscope (Zeiss Microscopy, South Africa) at a magnification of 40× to prevent any unintended mating. Distinguishing characteristics included the presence of black anal tufts on the hind tibiae in males, which are absent in females (Gilligan et al., 2011).

2.2.2 Release of sterile *T. leucotreta* into the laboratory cages

The laboratory cage studies were conducted following the methodology outlined by Hofmeyr et al. (2005) in a controlled environment (CE) room at approximately 26±1°C, 70±5% relative humidity (RH), and photoperiod of 16:8 (L:D) h. A total of 15 foldable insect-rearing cages (40 cm × 40 cm × 60 cm) were used. The moths were released into the cages, which contained 35 ripe Washington Navel oranges each. Before release into the cages, the adult sterile and fertile *T. leucotreta* were sorted into different ratios (Table 2.1). This grouping was done one day before the actual release, with male and female *T. leucotreta* being released on opposite sides of the cage. The selection of treatments within the cages was randomised, with each treatment being replicated three times, and the experiment repeated three times. Petri dishes with wet cotton wool were placed in each cage to provide water for the moths. Throughout the experiment period, the insects were allowed to mate and lay eggs without any disturbance. The experiment was conducted for approximately four weeks, thereafter, the fruit were thoroughly examined for any *T. leucotreta* infestation (brown larval penetration holes/spots with frass). Any fruit showing *T. leucotreta* infestation signs were identified and removed. The total number of damaged fruit (with larval penetration holes/spots) per cage and the number of larval entries (penetration holes/spots) per fruit were recorded for each treatment.

Table 2.1: Randomly assigned treatments to 15 insect-rearing cages to examine the effect of release ratios of sterile (S) to fertile (F) *T. leucotreta* on the incidence of fruit damage and the sterile male's competitiveness.

Treatment	Release ratio S: F	Number of sterile *T. leucotreta*		Number of fertile *T. leucotreta*		
		S male	S female	F male	F female	Total moths
A	0:1(Control)	0	0	10	10	20
B	10:1	100	100	10	10	220
C	20:1	200	200	10	10	420
D	40:1	400	400	10	10	820
E	60:1	600	600	10	10	1220

2.2.3 Determination of F1 sterility

The damaged fruit were placed in individual 500-ml plastic containers (10 cm × 8 cm) with mesh lids and incubated. To provide the larvae with suitable cocooning sites, pieces of cotton wool were placed inside each container. Rapidly decaying fruit were cut open, and any larvae found were carefully transferred into diet jars (13 cm × 7cm) (one diet jar per damaged fruit) to enable the larvae to complete their development (see Moore et al., 2014). Pupae collected from cotton wool and diet jars were transferred to individual 90-ml clear plastic containers (5 cm × 5 cm) and allowed to eclose.

After sexing, all the emerged adults (F1 generation) were coupled with fertile *T. leucotreta* adult counterparts of the opposite sex from XSIT. The pairs were placed in 90-ml clear plastic containers with snap-on lids, perforated to hold a moistened cotton dental wick, and placed in the CE room for them to copulate. Copulation between the pairs was allowed to occur followed by egg-laying on the inner sides of the containers until the death of the females (~8-10 days). Afterward, the total number of eggs (fecundity) laid in the containers was counted and recorded. After five days, the number of neonates that hatched (fertility) were counted and recorded from each container.

In the release ratio trials, the percentage of egg hatch was used to categorise the parentage of the F1 male, or female reared from the damaged fruit. If the percentage of egg hatch was < 5%, the F1 adult was designated as the progeny of a sterile male (SM) and fertile female (FF), and if the egg hatch was > 5%, the F1 was designated as the progeny of a fertile

male (FM) and an FF (Bloem et al., 2003). In these trials, it was assumed that sterile females (SF) were completely sterile, and as such, produced no F1 progeny (Bloem et al., 2003). The per generation rate of increase occurring from the P1 to the F1 generation in each cage was calculated by dividing the number of F1 male and female progeny produced by a fertile (FM × FF) mating by the number of P1 FM (10) and FF (10) released into each cage.

2.2.4 Statistical analysis

Data collected from the experiment were checked for homogeneity of variance (F test, Levene's test) and the residual deviations for non-normality were determined using the Shapiro Wilk test, revealing that the data were not normally distributed (Shapiro & Wilk, 1965). The data were analysed using a negative binomial generalised linear model analysis as an extension of the Poisson distribution to allow for count data with a significant proportion of zero values, with different ratios as the sources of variation, as recommended by O'Hara and Kotze (2010). Analysis of deviance (log-likelihood ratio statistic) was used to assess the goodness of fit of the Poisson regression model, which has a distribution similar to that of chi-squared ($\chi 2$). The dependent variables used in the statistical model included the number of larval entries, the number of damaged fruit, and the number of F1 *T. leucotreta* adults emerging from the damaged fruit from the different treatments. The data were also sorted by release ratios, where Spearman's rank correlation analysis was used to examine the relationship between the number of sterile *T. leucotreta* released into each cage and the number of larval entries, the number of damaged fruit, and the number of F1 adult *T. leucotreta* emerging from each treatment.

The model was used to analyse data from the number and percentage of hatched eggs laid by the F1 moths (that emerged from damaged fruit and were crossed with fertile adults of the opposite sex), where the F1 adult sex and cage treatment were the source of variation. Similarly, the model was also used to analyse the percentage of F1 males and females that were fathered by a fertile male, where the cage treatment was the source of variation. Differences among treatment means were separated using the Tukey-Kramer statistic ($P \leq 0.05$) for multiple comparisons when the statistical model indicated significant treatment effects. All analyses were performed in R. 4.2.2 (R Core Team, 2023).

2.3 Results

2.3.1 Fruit damage, larval entries, and F1 progeny

The ratio of sterile to fertile *T. leucotreta* assigned to the laboratory cages had a significant effect on the mean number of damaged fruit ($\chi 2 = 198.06$; df = 4; $P < 0.05$) (Fig. 2.1), the mean number of larval entries ($\chi 2 = 111.29$; df = 4; $P < 0.05$) (Fig. 2.2A), and the mean number of emerged F1 *T. leucotreta* adults ($\chi 2 = 41.43$; df = 4; $P < 0.05$) (Fig. 2.2B). There was a significant decrease in the number of damaged fruit and larval entries between the control and all treatments ($P < 0.05$) (Fig. 2.1; 2.2A). In addition, significantly lower mean number of damaged fruit and larval entries were recorded in 40:1 compared to 20:1. The highest percentage of damaged fruit (98.40 ± 4.20%) and the mean number of larval entries (397.70 ± 64.80 in 35 fruit per treatment replicate) were recorded from the control, while the lowest (37.50 ± 2.26% of fruit damaged; 33.10 ± 5.70 larval entries in 35 fruit per the treatment replicate) were recorded in 40:1 cages (Fig. 2.1; 2.2A). However, no significant differences in damaged fruit and larval entries were recorded between 10:1 and 20:1 as well as 10:1 and 60:1 (Fig. 2.1; 2.2A). The number of F1 progeny decreased with an increase in ratios (Fig. 2.2B). The highest mean number of F1 progeny was recorded in control (46.89 ± 9.76) while the lowest number was recorded in 60:1 (8.10 ± 1.90) (Fig. 2.2B). There was a significantly lower mean number of F1 progeny between control and 20:1, 40:1, and 60:1. However, there was no significant difference in the F1 progeny between control and 10:1.

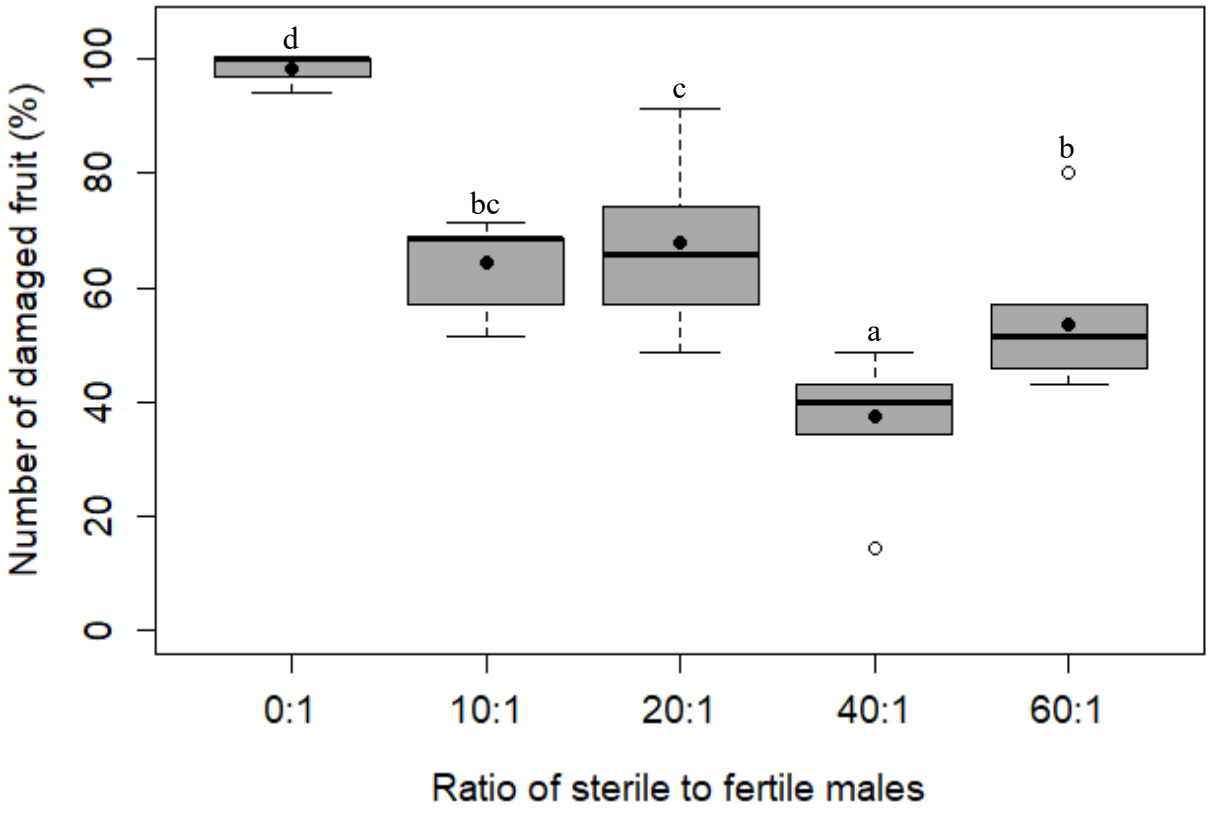
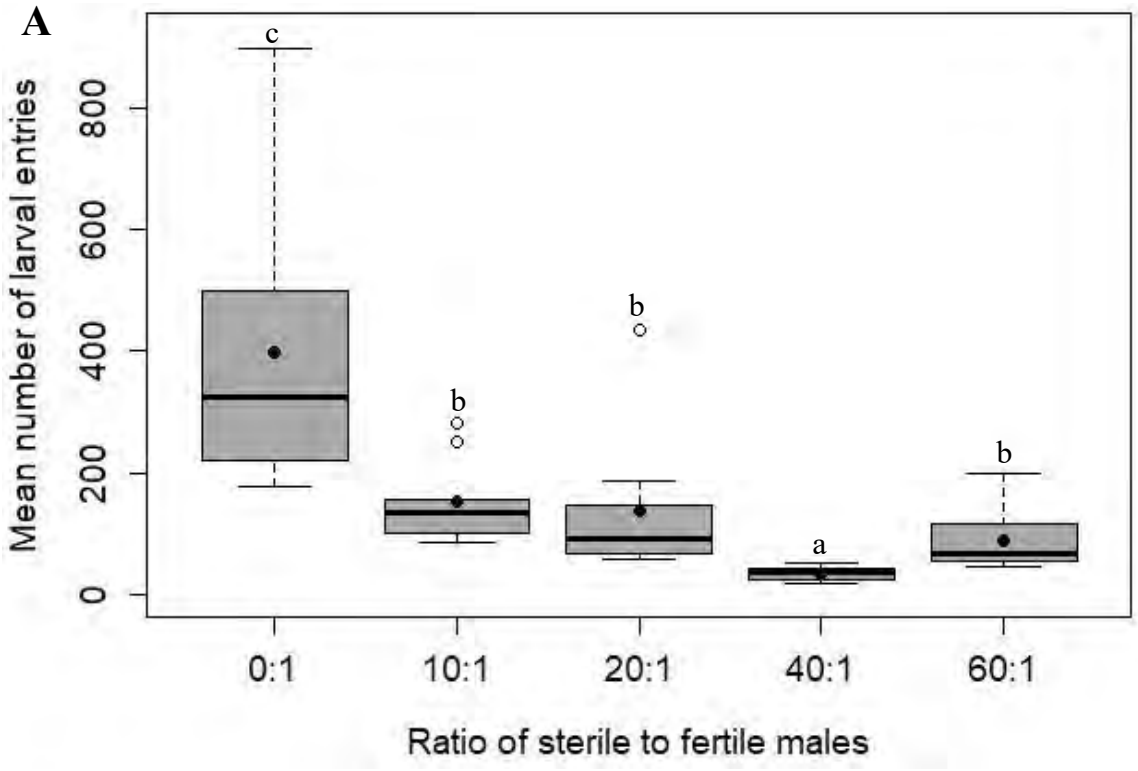


Fig. 2.1: The mean number of damaged fruit from different ratios of sterile to fertile males. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots indicate the mean number of damaged fruit per treatment, while the white dots represent the outliers. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).



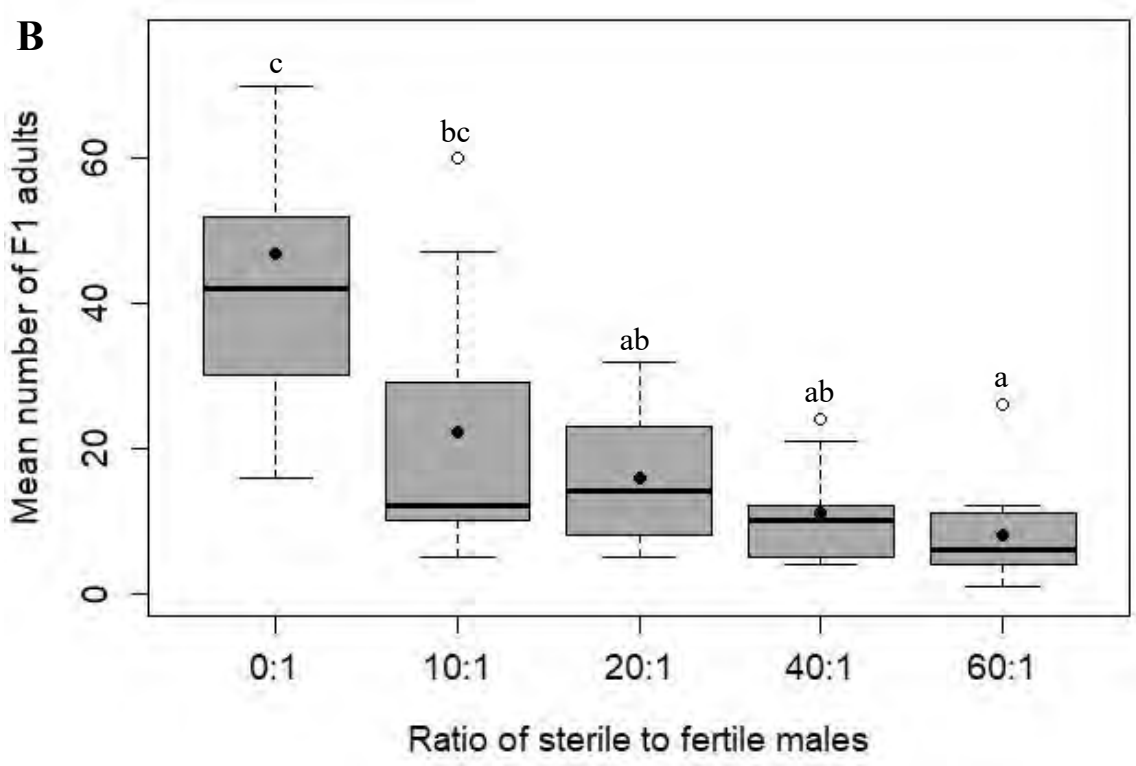
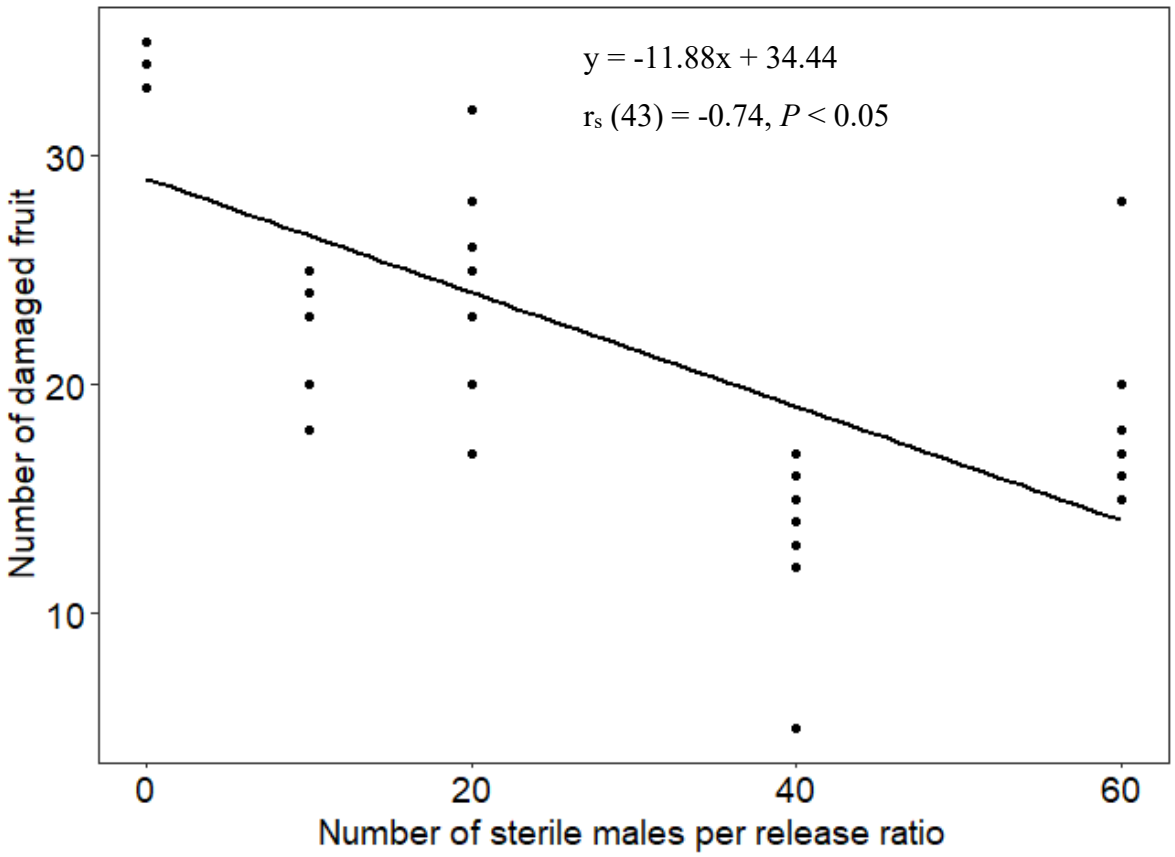


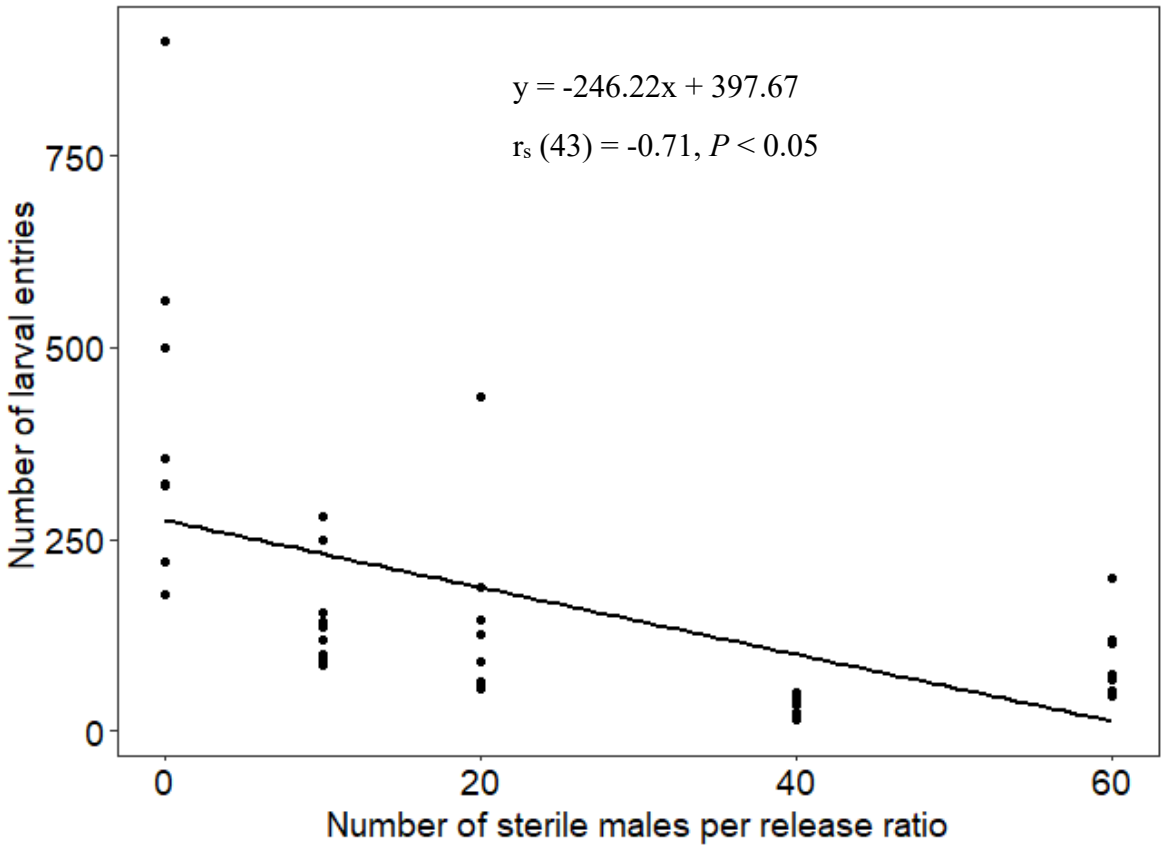
Fig. 2.2: The mean number of larval entries **A**, and F1 *T. leucotreta* adults **B** from different treatment ratios of sterile to fertile *T. leucotreta* males. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots indicate the mean number of larval entries and F1 *T. leucotreta* adults per treatment, while the white dots represent the outliers. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

The Spearman's rank correlation revealed a significant linear relationship between the number of sterile *T. leucotreta* adults released into each cage and the number of larval entries, the number of damaged fruit, as well as the number of F1 adults emerging from damaged fruit. There was a negative correlation between the number of damaged fruit and the number of sterile to fertile *T. leucotreta* in the cages ($P < 0.05$) (Fig. 2.3A). Likewise, the number of larval entries per treatment decreased as the number of sterile to fertile *T. leucotreta* in the cages increased ($P < 0.05$) (Fig. 2.3B). Moreover, as the number of sterile to fertile *T. leucotreta* in the cages increased, there was a decrease in the mean number of F1 *T. leucotreta* adults per treatment ($P < 0.05$) (Fig. 2.3C).

A


y = -11.88x + 34.44
rs (43) = -0.74, P < 0.05
Number of damaged fruit
30
20
10
0
20
40
60
Number of sterile males per release ratio

B


y = -246.22x + 397.67
r_s (43) = -0.71, $P < 0.05$
Number of larval entries
750
500
250
0
0
20
40
60
Number of sterile males per release ratio

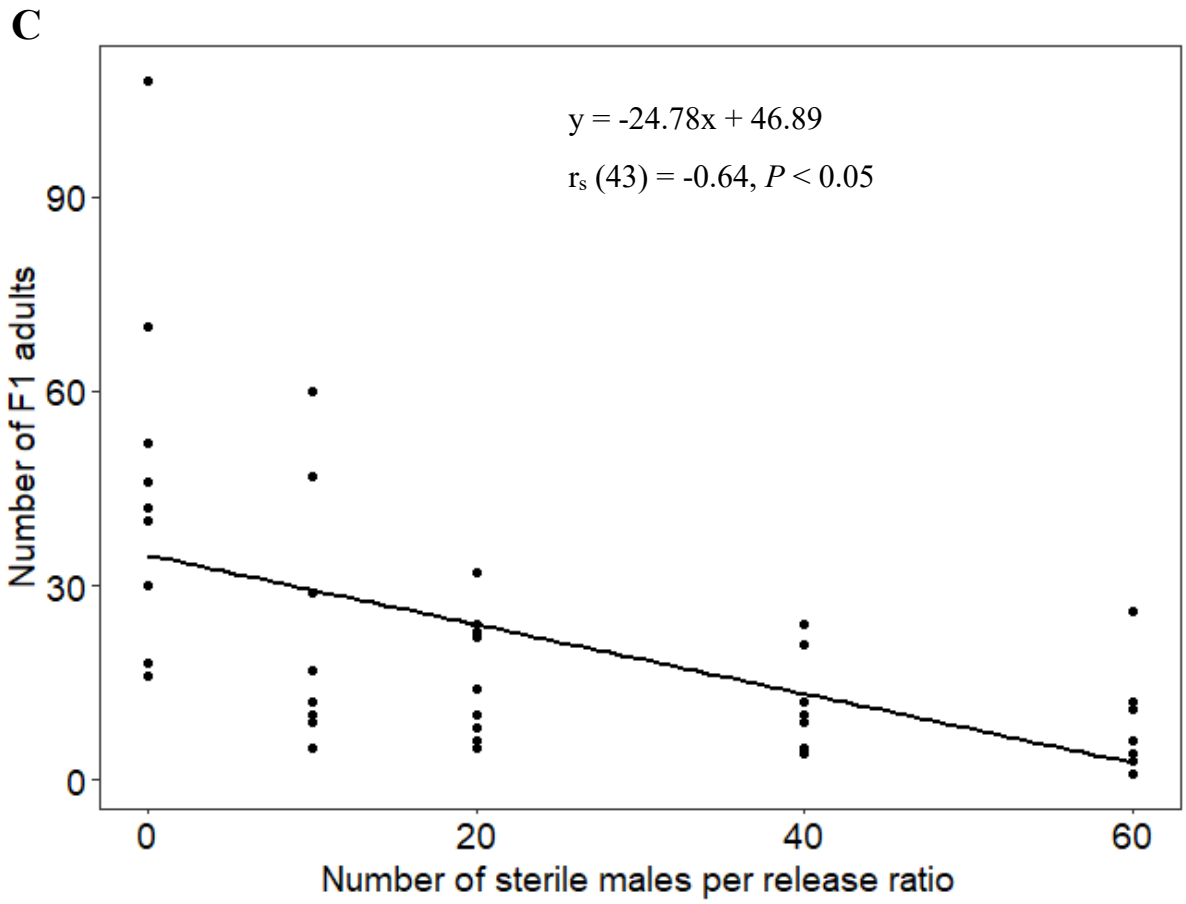


Fig. 2.3: A negative correlation was observed among the number of damaged fruit **A**, the number of larval entries **B,** and the number of emerged F1 adults **C** as the number of sterile to fertile male *T. leucotreta* increased per release ratio.

2.3.2 Fecundity and fertility

The mean number of eggs laid (fecundity) from F1 moths emerging from the damaged fruit and paired with fertile *T. leucotreta* of the opposite sex was significantly influenced by the ratio of sterile to fertile males ($\chi 2 = 4233.2$; df = 4; $P < 0.05$), the sex of F1 adults ($\chi 2 = 4663.6$; df = 1; $P < 0.05$), and the interaction between the ratio of sterile to fertile males and the sex of F1 adults ($\chi 2 = 1278.6$; df = 4; $P < 0.05$) *T. leucotreta* (Table 2.2). There was a significant difference in fecundity between F1 female and male crosses between the control and all the other treatments (Table 2.2). The highest fecundity from F1 female and male crosses was recorded for the control, while the lowest fecundity from both crosses was recorded for the 40:1. Significant higher fecundity of F1 female compared to F1 male crosses was observed in 10:1 and 20:1. However, 40:1 and 60:1 showed significant lower fecundity of F1 female compared to F1 male crosses (Table 2.2). However, no significant difference in

fecundity was recorded between F1 female and F1 male crosses from the control. Similarly, the percentage fertility from F1 moths emerging from damaged fruit and paired with fertile *T. leucotreta* of the opposite sex was significantly influenced by the ratio of sterile to fertile males ($\chi 2 = 1299.44$; df = 4; $P < 0.05$), the sex of F1 adults ($\chi 2 = 912.70$; df = 1; $P < 0.05$), and the interaction between the ratio of sterile to fertile males and the sex of F1 adults ($\chi 2 = 504.55$; df = 4; $P < 0.05$) (Table 2.2). The control had the highest mean percentage fertility from crosses involving F1 females than F1 males compared to other treatments. Similarly, the cages receiving different treatments of sterile to fertile ratios had significant differences in the percentage fertility involving crosses from F1 females and F1 males with fertile *T. leucotreta*, except treatment 10:1 (Table 2.2).

The ratio of sterile to fertile *T. leucotreta* per cage significantly influenced the mean number of fertile (= progeny of unsterile males) F1 male *T. leucotreta* ($\chi 2 = 25.68$; df = 4; $P < 0.05$) and F1 female *T. leucotreta* ($\chi 2 = 54.01$; df = 4; $P < 0.05$) emerging from the damaged fruit (Table 2.3). The mean number of fertile F1 males and females decreased with an increase in ratio such that control recorded the highest number while 60:1 recorded the lowest (Table 2.3). There were significant differences in the mean number of fertile F1 males and females between the control and all the other ratios. However, there were no significant differences in mean number of fertile F1 males and females between ratios 10:1 and 20:1 as well as 40:1 and 60:1. A comparison of fertile F1 males and females showed no mean significant differences across all treatments (Table 2.3). Similarly, the P1-F1 reproductive rate of increase decreased with an increase in ratio (Table 2.3). The ratio 60:1 had the lowest per generation rate of reproductive increase, while control recorded the highest increase from the P1 to the F1 generation. This treatment 60:1 resulted in a mean rate of increase <1 for both males (0.56×) and females (0.24×). From this, the mean per generation rate of reproductive increase for the fertile males and females was (0.4×), a value resulting in a slight decline from P1 to the F1 generation (Fig. 2.4).

Table 2.2: Effect of different ratios of sterile and fertile *T. leucotreta* released per cage, and the sex of F1 *T. leucotreta*, on the mean fecundity and fertility of F1 moths emerging from damaged fruit paired with fertile *T. leucotreta* of the opposite sex.

Cage treatment	Mean fecundity ± SE		Mean fertility ± SE (%)	
	F1 female	F1 male	F1 female	F1 male
0:1(Control)	166.60 ± 1.02g	128.00 ± 2.89g	55.40 ± 0.59f	30.30 ± 0.43d
10:1	98.50 ± 1.73f	71.20 ± 1.06d	28.20 ± 0.94c	24.80 ± 0.63c
20:1	103.60 ± 1.55f	58.50 ± 1.37c	36.40 ± 0.92e	25.80 ± 0.91c
40:1	31.70 ± 1.26a	44.30 ± 1.20b	13.00 ± 0.81a	18.40 ± 0.77b
60:1	42.00 ± 2.16b	83.40 ± 2.44e	11.60 ± 1.34a	32.50 ± 1.52de

Means within each column followed by the same letter are not significantly different ($P \geq 0.05$).

Table 2.3: Effect of different ratios of sterile and fertile *T. leucotreta* on the mean number of fertile F1 *T. leucotreta* adults emerging from fruit removed from the cages and the rate of increase for the P1-F1 generation.

Ratios	Mean ± SE fertile moths (progeny of unsterile males)		P1-F1 reproductive rate of increase	
	F_1 male	F_1 female	Male	Female
0:1	23.22 ± 4.96c	23.67 ± 5.16c	2.32×	2.36×
10:1	13.89 ± 3.07b	8.22 ± 1.95b	1.38×	0.82×
20:1	9.44 ± 2.17b	6.56 ± 1.60b	0.94×	0.65×
40:1	7.11 ± 1.69a	4.00 ± 1.06a	0.71×	0.40×
60:1	5.67 ± 1.39a	2.44 ± 0.73a	0.56×	0.24×

Means within each column followed by the same letter are not significantly different ($P \geq 0.05$).

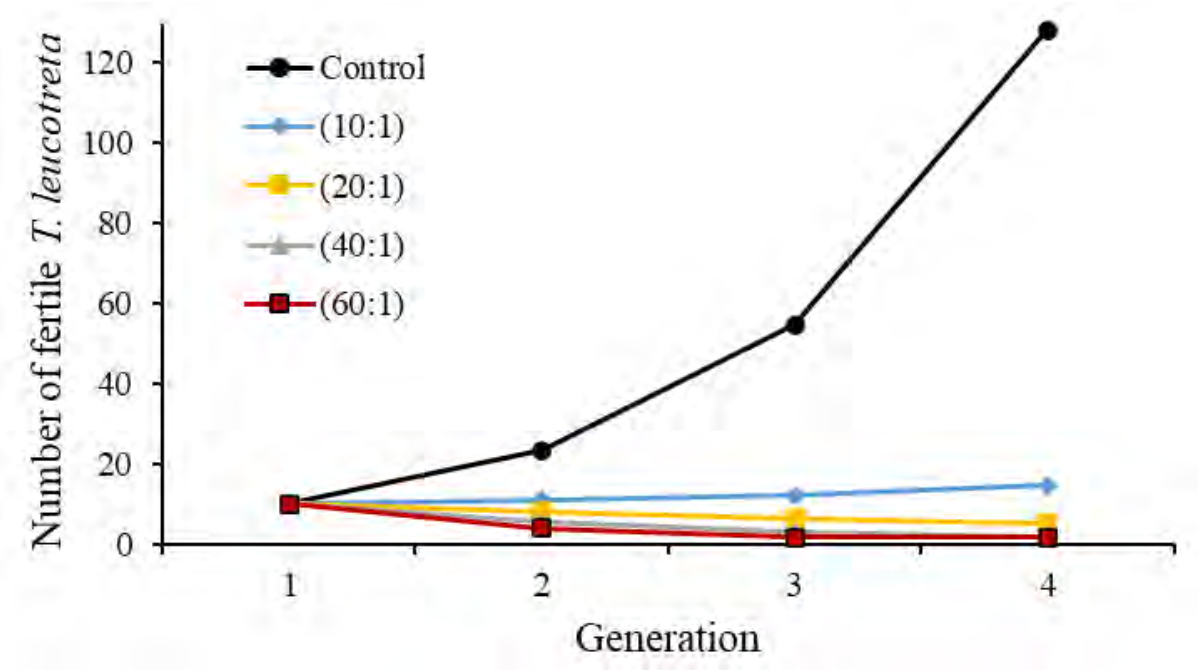


Fig. 2.4: Comparison of the estimated increase in the number of fertile *T. leucotreta* in a control population and treatment populations subjected to releases of sterile *T. leucotreta*. The first generation in the control population started with 10 pairs of fertile (F) *T. leucotreta* males (0:1), with a reproductive rate of 2.34× per generation. The treatment population 60:1 (= with the lowest per generation rate of increase) started generation 1 with 10 pairs of fertile *T. leucotreta*, with a release of 600 pairs of sterile (S) *T. leucotreta* males at the onset of each of the three generations (60:1), which reduced the reproductive rate to 0.4× per generation.

2.4 Discussion

An adequate OFR is one of the essential requirements for the successful implementation of a SIT programme. For instance, Klassen (2005) discussed the importance of "substantial overflooding ratios", while Lance & McInnis (2005) emphasised the significance of "a sufficiently high 'overflooding' ratio (sterile: fertile insect)" for the success of SIT. Likewise, Steiner (1969) demonstrated that eradication through SIT can be achieved only with a consistent overflooding of the native population. Despite this, there has been minimal effort to precisely connect OFR with the effectiveness of SIT. For example, Villaseñor et al. (2000) indicated that an 80:1 ratio was the lowest OFR required for controlling *C. capitata*, while Steiner (1969) suggested a 20:1 ratio as crucial for eradicating populations of *B. dorsalis*, *C. capitata* and *Zeugodacus cucurbitae*. Nevertheless, neither study offered substantial evidence on these proposed critical OFR values.

Conversely, some mathematical models have been created to explicitly determine the minimum OFR required to inhibit the reproduction of the target population. These models, first introduced by Knipling (1955, 1959), essentially alter geometric population growth by incorporating factors such as the count of sterile and fertile males in the population (indicative of infertile matings) and a coefficient representing the competitive ability of sterile males, which adjusts their numerical prevalence (Berryman, 1967; Barclay, 2005). Despite their accuracy, these models include variables like male and female population size and the population's inherent rate of growth, which are challenging to gauge in real-world settings. As a result, modelling strategies are not greatly employed in the establishment of specific OFR targets for field SIT programmes targeting tephritid and tortricid pests (Barclay, 2005).

In this study, we explored the impact of different ratios of sterile to fertile *T. leucotreta* adults on fruit damage and the sterile male competitiveness in the treatments. The findings revealed a consistent decrease in the mean number of damaged fruit, larval entries, and F1 *T. leucotreta* adults that were recorded across all treatments receiving sterile *T. leucotreta*, in contrast to the cages hosting fertile *T. leucotreta*. These results closely align with reports by Hofmeyr et al. (2005), suggesting that the sterile males released per cage demonstrated competitive ability, effectively inducing high levels of sterility in their fertile counterparts within the cages (Bakri et al., 2021). Consequently, they efficiently transferred sterile spermatophores to the fertile *T. leucotreta* resulting in the production of non-viable eggs with a low hatchability (LaChance, 1985). Therefore, this resulted in the reduction of fruit damage and, ultimately, fewer F1 progeny, due to their reduced reproductive capacity, thereby potentially aiding in pest population suppression (Bloem et al., 2003). Similarly, our results support findings by Hofmeyr et al. (2015) and Boersma (2021), indicating that the incidence of damaged fruit and fruit drop per tree was notably lower in Navel orange orchards under SIT compared to non-SIT orchards in a SIT pilot project.

The ratio of sterile to fertile *T. leucotreta* in the cage treatments led to a reduced number of F1 adults compared to the control. This suggests that the sterile *T. leucotreta* males released in the cages effectively outcompeted the fertile males for access to the fertile females. The findings corroborate with those of Hofmeyr et al. (2005), depicting that the mean number of fertile *T. leucotreta* males and females produced in the F1 generation was significantly lower in cages receiving sterile *T. leucotreta* as compared to control cages, resulting in a lower per generation rate of reproductive increase (from the P1 to the F1 generation) in these cages. These findings align with previous studies by Bloem et al. (1999)

on *C. pomonella* (L.) (Lepidoptera: Tortricidae), Hight et al. (2005) on *Cactoblastis cactorum*, Hofmeyr et al. (2005) on *T. leucotreta*, Flores et al. (2014) on *Anastrepha ludens* (Loew) (Diptera: Tephritidae), and by Shelly & McInnis (2016) focusing on *B. dorsalis*, *C. capitata* and *Z. curcubitae*. These studies collectively depict that the population growth of these respective pests can be influenced by the release of sterile conspecifics, even when the release ratios used are relatively low. However, increasing the ratio of sterile to fertile males (OFR) will not necessarily yield desired outcomes if released males, irrespective of their abundance, fail to meet a certain minimum threshold of "acceptable" courtship performance required by most or all wild females (Itô & Yamamura, 2005; Shelly & McInnis, 2016).

Our findings demonstrate the efficacy of sterile *T. leucotreta* adults in mitigating incidences of fruit damage and reducing the mean number of fertile F1 adults produced within a controlled environment. However, it is difficult to predict the potential impact of releasing sterile *T. leucotreta* against a fertile population unless the efficacy depicted in the laboratory cage trials is extrapolated for multiple generations (Hofmeyr et al. 2005). In the study, a simulation was conducted to emulate the real-world scenario of a SIT programme, wherein sterile *T. leucotreta* would be continuously released from the onset of a growing season when the population of fertile wild *T. leucotreta* would be at its lowest. The investigation compared the projected rate of reproductive increase in the number of fertile *T. leucotreta* within a "control" population against the number of fertile (unsterile) *T. leucotreta* within a population subject to releases of sterile *T. leucotreta* treated with a radiation dosage of 180 Gy.

In the control population, the first generation commenced with 10 pairs of fertile *T. leucotreta*, exhibiting an average reproductive increase rate of 2.34× per generation. Likewise, the treatment involving the sterile population started the first generation with 10 pairs of fertile (unsterile) *T. leucotreta* and received a release ratio of 600 pairs of sterile *T. leucotreta* irradiated with a dosage of 180 Gy at the onset of each of the three generations (equivalent to a ratio of 60: 1 for each generation) resulting in a reduced mean reproductive increase rate for both males and females of 0.4× per generation. According to the model, derived from data obtained from our laboratory cage trial, the population of fertile moths receiving treatment with sterile moths experienced a slight decline, whereas the number of fertile *T. leucotreta* in the control population increased by over 14%. Our findings align with those of Hofmeyr et al. (2005), demonstrating that treatment with a sufficient ratio of sterile to fertile *T. leucotreta* can significantly reduce the proliferation of a fertile population across several generations.

In summary, the laboratory study demonstrated that a ratio of 60:1 can significantly reduce the proliferation of the fertile population, leading to enhanced suppression of *T. leucotreta*. This ratio of sterile to fertile *T. leucotreta* males surpasses the current benchmark release ratio of 10:1 determined by Hofmeyr et al. (2005), in South Africa. Additionally, field trials conducted by Hofmeyr & Hofmeyr (2009, 2010) and Moore (2011) reported a 40:1 ratio under field conditions. Our findings confirm those of Hofmeyr et al. (2005), who recommended a 10:1 ratio, which has been used commercially since 2007 in controlling *T. leucotreta* in citrus orchards (XSIT, 2018). However, this ratio does significantly suppress the pest in controlled and confined environments. Our study also showed that any release ratio can have a suppressant effect on *T. leucotreta*, as it provides an opportunity for some fertile-sterile pairings, rather than fertile-fertile pairings. The findings from our present study suggest that a ratio higher than 10:1, particularly at 40:1 and 60:1, can further enhance the efficacy of SIT. Nevertheless, additional research is necessary to thoroughly evaluate the effectiveness and feasibility of these findings under real-world field conditions. The findings of our study support the continued improvement of the efficacy and effectiveness of the SIT programme as a control and management strategy for *T. leucotreta* in South Africa.

CHAPTER 3

Effects of different combinations of sterile and fertile *Thaumatotibia leucotreta* (Meyrick) on fruit infestation and population growth rate

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3.1 Introduction

Thaumatotibia leucotreta is a key agricultural pest endemic to SSA (Hattingh et al., 2020) and was first recorded in citrus in South Africa in 1899 in KwaZulu-Natal Province (Newton, 1998; Bloem et al., 2007; Hofmeyr et al., 2015; Boersma, 2021). The pest is extremely polyphagous with an extensive host range of cultivated plants including economically important crops such as macadamia, peppers, pomegranates, stone fruit, and most citrus cultivars (Newton, 1998; Moore, 2002; Bloem et al., 2003; EPPO, 2013; Moore & Manrakhan, 2022; EFSA, 2024), as well as wild plant species (Kirkman, 2007; EPPO, 2013; EFSA, 2024). *Thaumatotibia leucotreta* is a key pest of citrus in southern Africa, particularly Navel oranges (Newton, 1998; Grout & Moore, 2015; EFSA, 2024). The damage inflicted by *T. leucotreta* involves larval penetration of the fruit and pulp feeding, resulting in fruit drop. Detection of fresh larval infestation requires thorough inspection (Moore, 2022). When the fruit is green, the area around the penetration point assumes a yellow hue, whereas, in ripe fruit, it initially turns orange, then a brown hue with a sunken appearance as the infested tissue decomposes (Moore, 2022). Consequently, fruit abscission occurs within three to five weeks after infestation (Grout & Moore, 2015). Where left uncontrolled or where the effective biocontrol complex is disrupted, crop losses can be significant (Newton, 1998; Moore, 2002). Most importantly, stringent quarantine and import protocols have been implemented by export markets such as the USA, People's Republic of China, Republic of Korea, and most recently, the European Union, to prevent live specimens of this phytosanitary pest from entering these regions (USDA, 2010; SA-DAFF, 2015; EU, 2017; EFSA, 2024).

Enhancing control methods is a vital strategy for effectively managing pest populations below economic injury levels, particularly when it comes to *T. leucotreta* in citrus orchards (Moore & Hattingh, 2012; Moore, 2022). Consequently, control measures before exporting must be exemplary as any fruit bearing brown larval penetration holes/spots with frass is regarded as damaged and unsuitable for export (Moore, 2022). Since 2007, SIT has been practiced in major citrus-growing regions in South Africa (Hofmeyr et al., 2005; Hofmeyr et al., 2015; Boersma, 2021). This method has proven highly effective in suppressing *T. leucotreta* populations. In South Africa, treated *T. leucotreta* are reared and sterilised at XSIT in Citrusdal, Western Cape Province, before being transported in a cold, immobilised state, using refrigerated trucks, to release sites (Barnes et al., 2015; Boersma & Carpenter, 2016). During this immobilised state, the cold treatment can negatively impact their fitness, reducing their ability to compete successfully for mating with their wild

counterparts in the orchards (Nepgen et al., 2015). This situation is even more problematic because the ionising radiation used for sterilisation causes oxidative stress (Calabrese et al., 2013). However, for SIT to be successful, the treated male moths must compete successfully with fertile wild males for wild females, to induce sterility in the fertile population (Boersma, 2021).

To implement a successful SIT programme, it is crucial to make well-informed decisions regarding the appropriate radiation dose required to induce sterility in the natural population, (Bakri et al., 2005; Robinson, 2006). The shape of the dose-response curve, determined by assessing fertility in insects exposed to escalating radiation doses, reflects the nature of initial chromosomal lesions (Robinson, 2006). In the *T. leucotreta* SIT programme, Bloem et al. (2003) investigated the impact of increasing radiation doses on mature pupae and newly emerged adults of both sexes, which were then crossed with fertile counterparts. The study found that a radiation dose of 200 Gy completely sterilised adult *T. leucotreta* females when mated with untreated males. Conversely, newly emerged males treated with 350 Gy retained a residual fertility of 5.2% when mated with untreated females, while males treated as pupae had a residual fertility of 3.3% (Bloem et al., 2003). This led to F1 sterility due to increasing radiation doses applied to P1 males, resulting in reduced fecundity and fertility, increased F1 mortality during development, and a male-biased sex ratio (Bloem et al., 2003). Following this, Hofmeyr et al. (2005) used newly emerged adult *T. leucotreta* treated with either 150 or 200 Gy and released them into field cages at ratios of 5:1 or 10:1 to determine the effective release ratio. The study showed that higher overflooding ratios decreased larval damage and F1 progeny. Additionally, significant reductions in egg hatch were observed in the progeny of crosses between F1 females or F1 males originating from the treatment cages compared to those from control cages. Similarly, this is congruent with our findings in Chapter 2, where the 60:1 ratio had the lowest mean number of F1 progeny (see "fruit damage, larval entries, and F1 progeny" in the 2.3 Results section above). The 150 Gy and 10:1 ratio treatment produced the lowest mean number of fertile F1 adult females and males, establishing this as the benchmark ratio to be used (Hofmeyr et al., 2005). The radiation dose, which fluctuates between 150 and 200 Gy depending on season, is used to ensure that the fitness of male sterile *T. leucotreta* is maintained, thus remaining competitive against their wild counterparts (Bloem et al., 2003; Boersma, 2021).

Regular monitoring of the mating dynamics and compatibility between untreated and treated insects is essential. This is because there is a possibility that genetic changes occurring in laboratory-reared colonies can lead to alterations in reproductive mechanisms

viz, pheromone emission, detection, and mating behaviour as suggested by Whitten and Mahon (2005). For instance, research conducted by Hiblno and Iwahashi (1991) demonstrated that female melon flies, *Z. cucurbitae*, on Okinawa Island were less inclined to mate with treated males compared to wild males. Similarly, Weldon (2005) discovered that mass-reared male Queensland fruit flies, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), modified their calling and mating behaviour due to unnatural selection pressures in a laboratory environment, although, resistance to treated insects by wild individuals is exceedingly rare and has not been documented as significant in any SIT programme (Whitten & Mahon, 2005). Where possible, male-only SIT releases are often considered preferable, such as in *C. capitata* through a genetic sexing strain, as it is feasible and economical from a production standpoint (Rendón et al., 2004). For example, in Medfly SIT programmes, male-only releases are conducted, as treated Medfly females can still damage fruit through attempted oviposition, even if eggs are not laid, i.e. 'blind stings' (Rendón et al., 2004; Hofmeyr et al., 2015). This is also imperative in programmes aiming at reducing pest populations that are hematophagous and vectors of diseases, such as malaria, *Anopheles gambiae* (Meigen) (Diptera: Culicidae) and sleeping sickness, *Glossina* spp., or where females cause damage to the target crop (Calkins & Parker, 2005).

Even though releasing only treated males is typically considered sufficient in a SIT programme, due to the impracticality of sex-separating treated *T. leucotreta* in SIT programmes, dual-sex releases are conducted (Marec & Vreysen, 2019; Boersma, 2021). Treated females can either create positive or negative sperm sinks, depending on whether they mate with untreated or treated males, respectively, thereby influencing the rate of population suppression (Calkins & Parker, 2005). A positive sperm sink, where treated females attract wild fertile male sperm, is preferred. When this occurs, fruit infestation from treated and wild fertile *T. leucotreta* might be reduced, hence improving the effectiveness of the technique. Since the release of treated male and female *T. leucotreta* resulted in a significantly lower overall mean egg number and hatch than a male-only sterile release (van Steenderen, 2017), it is important to understand the impact of different combinations of treated and untreated male and female *T. leucotreta* on fruit infestation and per generation rate of population growth in laboratory cage studies. Therefore, the study investigated whether different combinations of treated and untreated male and female *T. leucotreta* can affect fruit infestation, larval entries, and population growth rate in laboratory cages. The results obtained are discussed in the context of improving the effectiveness of SIT in the suppression of *T. leucotreta* through the release of different combinations of treated and

untreated *T. leucotreta* in an area-wide sterile insect release programme in citrus orchards in South Africa.

3.2 Materials and Methods

3.2.1 Test insects

Adult sterile and fertile *T. leucotreta* were sourced from XSIT. To render them sterile, the moths were exposed to 180 Gy of gamma irradiation. Subsequently, the moths were transported to Rhodes University, Makhanda, South Africa (~840 km, 13-14 h) in a polystyrene cooler box with dry ice bricks, maintaining a temperature of 4-6°C. This controlled temperature was crucial in minimising the moths' activity and preventing mating while in transit (Nepgen et al., 2015). The moths were approximately 48h old upon arrival and they were kept in a refrigerator at a temperature of 5°C and were immediately sorted by sex using a stereomicroscope (Zeiss Microscopy, South Africa), at a magnification of 40× to prevent any unintended mating. Distinguishing characteristics included the presence of black anal tufts on the hind tibiae in males, which are absent in females (Gilligan et al., 2011).

3.2.2 Release of treated *T. leucotreta* into laboratory cages

Laboratory cage studies were conducted similarly to Hofmeyr et al. (2005), in a controlled environment (CE) room at 26±1°C, 70±5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. A total of 15 insect-rearing cages (40 cm × 40 cm × 60 cm) were used. The moths were released into the cages, each containing 35 ripe Washington Navel oranges. Before release into the cages, the treated and untreated *T. leucotreta* were sorted into different combinations (Table 3.1). This grouping was done 24h prior to the actual release, with male and female *T. leucotreta* being released on opposite sides of the laboratory cage. The treatments were randomly assigned to the cages, with each treatment being replicated three times, and the experiment repeated three times. Petri dishes with wet cotton wads were placed in each cage, to provide water for the moths. Throughout the experimental period, the insects were allowed to mate and oviposit without any disturbance. The experiment was conducted for 4 weeks. Thereafter, the fruit were thoroughly examined externally for any *T. leucotreta* damage signs (brown larval penetration holes/spots with frass). Any fruit with larval penetration holes/spots were regarded as damaged, and the total number of damaged fruit per cage was recorded. Similarly, the number of larval entries (penetration holes/spots) per fruit were recorded for each treatment.

Table 3.1: Randomly assigned treatments in 15 insect-rearing cages to examine the effect of different combinations of treated and untreated *T. leucotreta* on fruit damage and population growth.

Treatment	**1**		**2**		**3**		**4**		**5**			
Combination	UM×UF		TM×UF		UM×TF		TM×TF		UM×UF×TM×TF			
Untreated	10	10	10		10				10	10		
Treated				10		10	10	10			10	10

UM- untreated male, UF- untreated female, TM- treated males, TF- treated females. A ratio of 1:1 was used between treated and untreated *T. leucotreta* males and females.

3.2.3 Determination of F1 sterility

The apparently damaged fruit were placed in individual 500 ml round plastic containers (8 cm × 10 cm) with mesh lids. For the provision of suitable cocooning substrates for the larvae, wads of cotton wool were placed inside each container. Rapidly decaying fruit were cut open and any larvae found were carefully transferred into diet jars (13 cm × 7 cm) (one diet jar per damaged fruit). This was to enable the larvae to complete their development under optimum conditions. (see Moore et al., 2014). Pupae collected from cotton wool and diet jars were transferred to individual 90 ml clear round plastic containers (5 cm × 5 cm) and allowed to eclose.

After sexing, all the emerged adults (F1 generation) were crossbred with untreated *T. leucotreta* adults of the opposite sex sourced from XSIT. Each pair was placed in 90 ml clear round plastic containers with snap-on lids, perforated to hold a moistened cotton dental wick, and placed in the CE room for mating. Mating between the pairs was allowed to occur, followed by egg-laying on the inner sides of the containers until the death of the females (~8-10 days). Afterward, the total number of eggs laid (fecundity) per container per treatment was counted and recorded. After 5 days, the number of neonates that hatched (fertility) was counted and recorded per container.

In these combination trials, the percentage egg hatch was used to categorise the parentage of the F1 male, or female reared from the damaged fruit. If the percentage egg hatch was < 5%, the F1 adult was designated as the progeny of a treated male (TM) and untreated female (UF), and if the egg hatch was > 5%, the F1 was designated as the progeny

of an untreated male (UM) and a UF (Bloem et al., 2003). In these trials, it was assumed that treated females (TF) were completely sterile and as such, produced no F1 progeny (Bloem et al., 2003). The per generation rate of increase occurring from the P1 to the F1 generation in each cage was calculated by dividing the number of F1 male and female progeny produced by an untreated (UM × UF) mating by the number of P1 UM (10) and UF (10) released into the cages.

3.2.4 Statistical analysis

Data collected from the experiment were checked for homogeneity of variance (F test, Levene's test), and the determination of residual deviations for non-normality was done using the Shapiro-Wilk test (Shapiro & Wilk, 1965). The test revealed that the data were not normally distributed. A negative binomial generalised linear model analysis, as an extension of the Poisson distribution to accommodate for count data with a significant proportion of zero values, was employed to analyse the data with different treatments as the sources of variation, following a recommendation by O'Hara and Kotze (2010). Analysis of deviance (log-likelihood ratio statistic) was utilised to evaluate the goodness of fit of the Poisson regression model, which has a distribution akin to that of chi-squared ($\chi 2$). The dependent variables in the statistical model comprised the number of larval entries, the number of damaged fruit, and the number of F1 *T. leucotreta* adults emerging from the damaged fruit from the different treatments. Additionally, the model was used to analyse data on the number and percentage of hatched eggs laid by the F1 moths (resulting from damaged fruit and crossed with fertile adults of the opposite sex), with F1 adult sex and cage treatment as the sources of variation. Similarly, the model was applied to analyse the number of F1 males and females that were fathered by a fertile male, with the cage treatment as the source of variation. Differences among treatment means were separated using the Tukey-Kramer statistic ($P \leq 0.05$) for multiple comparisons when the statistical model indicated significant treatment effects. All analyses were conducted using R version 4.2.2 (R Core Team, 2023).

3.3 Results

3.3.1 Fruit damage, larval entries, and F1 progeny

The different combinations of sterile to fertile *T. leucotreta* significantly affected the mean number of damaged fruit ($\chi 2 = 109.31$; df = 4; $P < 0.05$) (Fig. 3.1), the mean number of larval entries ($\chi 2 = 59.90$; df = 4; $P < 0.05$) (Fig. 3.2), and the mean number of emerged F1 *T.*

leucotreta adults ($\chi 2 = 48.70$; df = 4; $P < 0.05$) (Fig. 3.3). The highest percentage of damaged fruit was recorded in the control cages (96.80 ± 16.66%), while the lowest was recorded in treatment TM×TF (13.33 ± 2.56% of damaged fruit) (Fig. 3.1). There was a significant decrease of 40.30 ± 7.12%, 31.10 ± 5.57% and 13.33 ± 2.56% of damaged fruit between the control and TM×UF, UM×TF and TM×TF combinations respectively (Fig. 3.1). The mean number of larval entries in the control cages (390.22 ± 134.62 larval entries per treatment replicate) was significantly higher than combinations TM×TF (0.00 ± 0.00 larval entries per treatment replicate), TM×UF (59.22 ± 20.57 larval entries per treatment replicate), and UM×TF (67. 89 ± 23.55 larval entries per treatment replicate) (Fig. 3.2). Likewise, the control cages exhibited a significantly higher mean number of emerged F1 adults (84.89 ± 32.15) relative to combinations TM×TF (0.00 ± 0.00 F1 adults per replicate), TM×UF (11.67 ± 4.54 F1 adults per replicate) and UM×TF (12.22 ± 4.75 F1 adults per replicate) (Fig. 3.3). However, no significant differences in the percentage of damaged fruit, mean number of larval entries, and emerged F1 adults were recorded between the control cages and combination UM×UF×TM×TF (Fig. 3.1; 3.2; 3.3). Furthermore, among cages receiving different combinations of treated and untreated *T. leucotreta*, significantly fewer damaged fruit, and larval entries were recorded in cage treatment TM×TF. Conversely, cages receiving treatment UM×UF×TM×TF showed a significantly higher percentage of damaged fruit (84.40 ± 14.57%), the mean number of larval entries (294.22 ± 101.54 per treatment replicate), and the emerged F1 adults (63.67 ± 24.15) than the cages receiving treatments TM×UF, UM×TF, and TM×TF (Fig. 3.1; 3.2; 3.3).

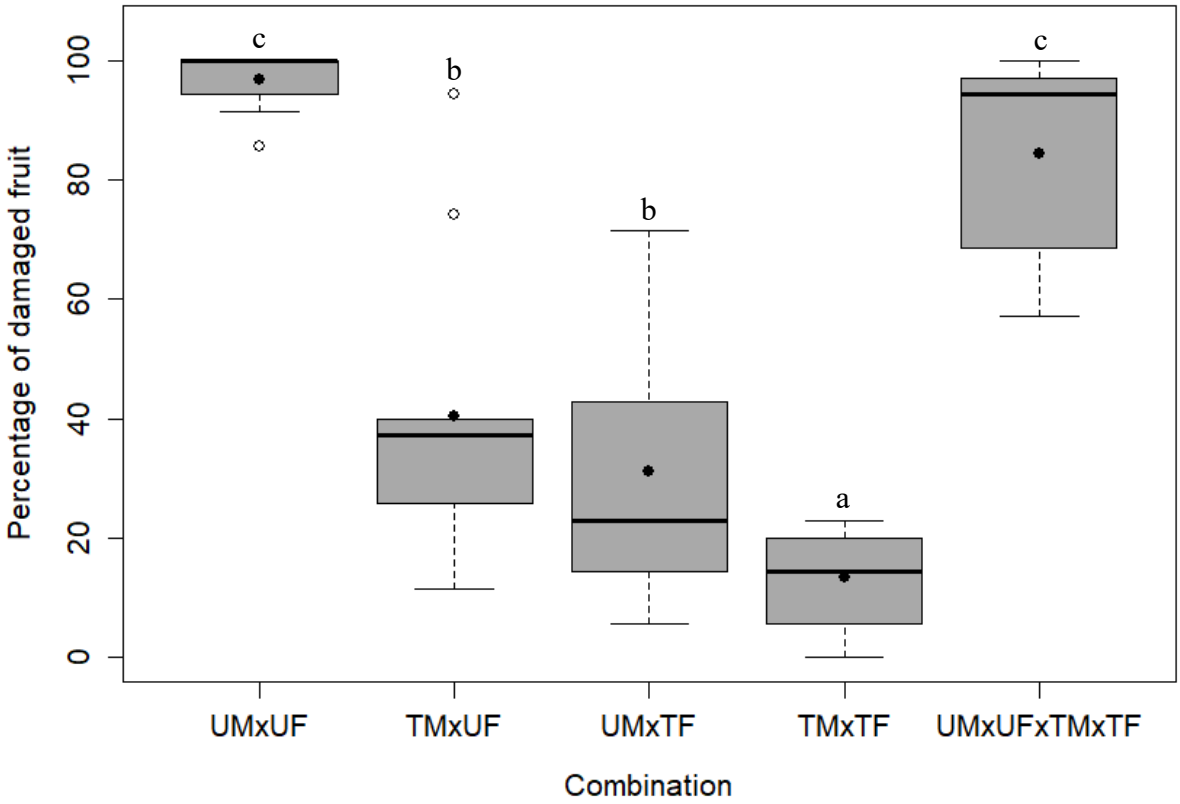

Fig. 3.1: The percentage of damaged fruit from different cages receiving different combinations of treated and untreated *T. leucotreta*. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots indicate the mean percentage of damaged fruit per treatment, while the white dots represent the outliers. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

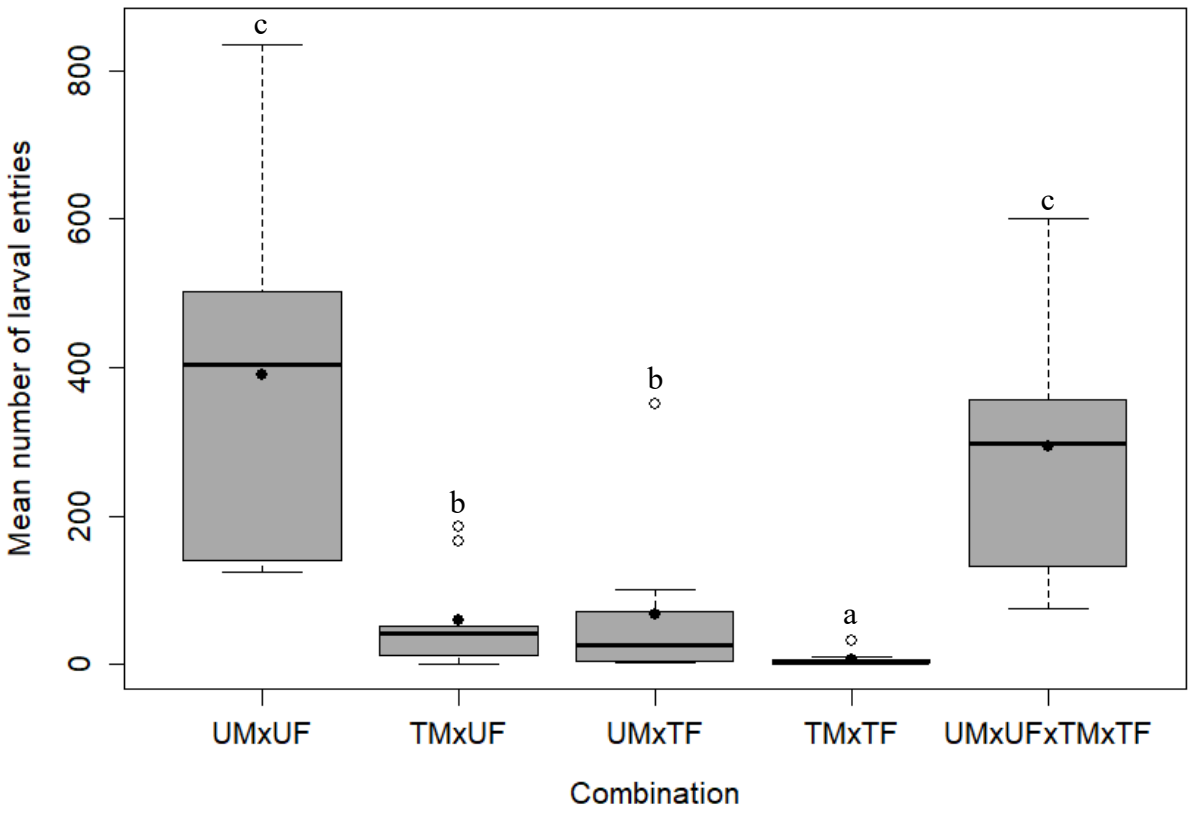

Fig. 3.2: The mean number of larval entries from different cages receiving different combinations of treated and untreated *T. leucotreta*. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots indicate the mean number of larval entries per treatment, while the white dots represent the outliers. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

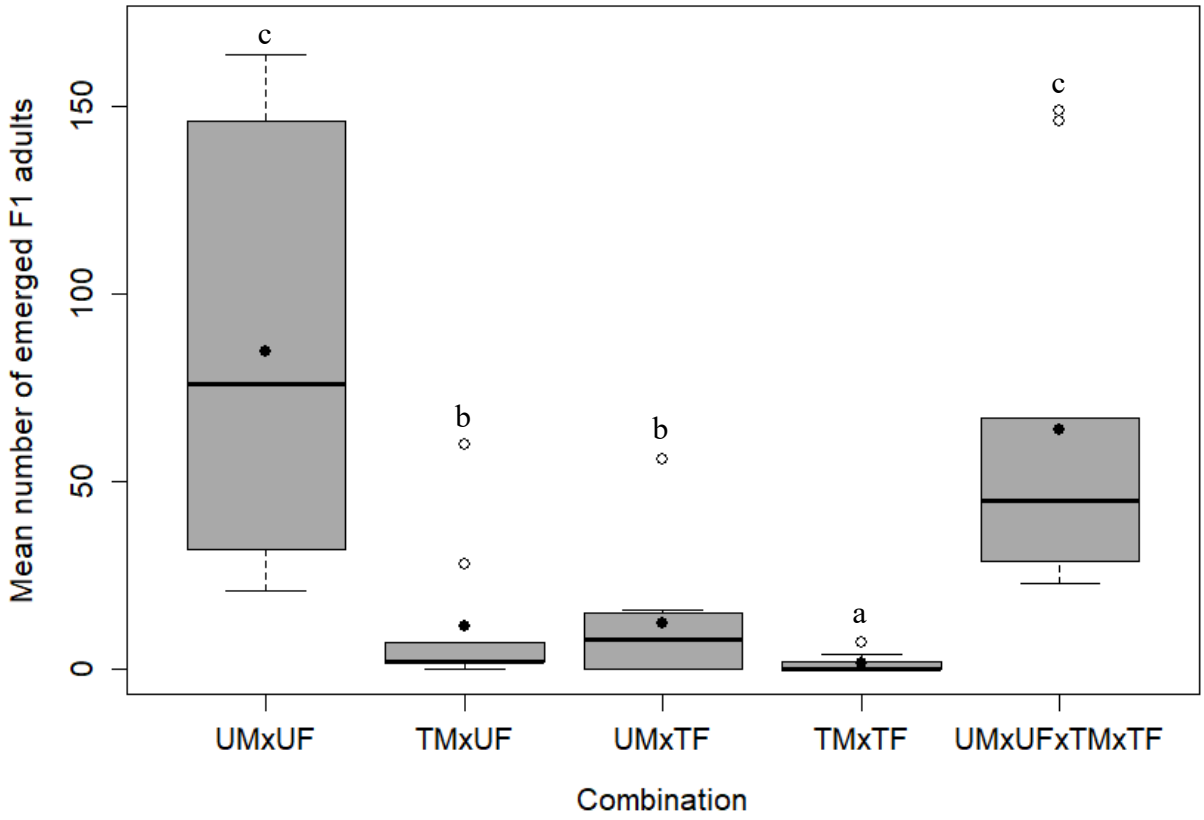

Fig. 3.3: The mean number of emerged F1 adults from different cages receiving different combinations of treated and untreated *T. leucotreta*. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots indicate the mean number of emerged F1 adults per treatment, while the white dots represent the outliers. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

3.3.2 Fecundity and fertility

The mean fecundity of F1 moths emerging from the damaged fruit and crossbred with fertile *T. leucotreta* of the opposite sex was significantly influenced by the different combinations of treated and untreated *T. leucotreta* ($\chi2 = 37.18$; df = 4; $P < 0.05$), the sex of emerged F1 *T. leucotreta* adults ($\chi2 = 38.65$; df = 1; $P < 0.05$), and the interaction between the different combinations of treated and untreated *T. leucotreta* and the sex of the emerged F1 *T. leucotreta* adults ($\chi2 = 14.54$; df = 3; $P < 0.05$) (Table 3.2). Similarly, fertility was significantly affected by the different combinations of treated and untreated *T. leucotreta* ($\chi2 = 66.05$; df = 4; $P < 0.05$), the sex of emerged F1 *T. leucotreta* adults ($\chi2 = 10.86$; df = 1; $P < 0.05$), and the interaction between the different combinations of treated and untreated *T. leucotreta* and the sex of the emerged F1 *T. leucotreta* adults ($\chi2 = 7.78$; df = 3; $P = 0.05$) (Table 3.2). There was a significant reduction in fecundity and fertility between controls and

TM×UF, UM×TF, and TM×TF from F1 females and F1 males' crosses. However, no significant differences in fecundity and fertility were recorded between controls and treatments UM×UF×TM×TF from crosses involving F1 females and F1 males (Table 3.2). Similarly, significantly fewer eggs were laid when crosses involved F1 males compared to crosses involving F1 females in cages receiving different combinations of treated and untreated *T. leucotreta*, except in combinations TM×TF and UM×UF×TM×TF.

Table 3.2: Effect of different combinations of treated and untreated *T. leucotreta* released per cage on the mean fecundity and fertility by F1 moths emerging from damaged fruit and crossbred with fertile *T. leucotreta* of the opposite sex.

Cage treatment	Mean fecundity ± SE		Mean fertility ± SE (%)	
	F_1 female	F_1 male	F_1 female	F_1 male
Control (UM × UF)	176.70 ± 8.96e	145.90 ± 7.62e	77.00 ± 2.24c	73.6 ± 2.21c
TM × UF	140.20 ± 15.21cd	64.60 ± 8.35b	65.10 ± 4.08b	49.60 ± 3.73b
UM × TF	144.00 ± 50.81cd	57.00 ± 19.53b	62.10 ± 9.96b	35.90 ± 7.32b
TM × TF	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
UM × UF × TM × TF	159.30 ± 9.41e	99.40 ± 6.62e	83.50 ± 33.02c	55.60 ± 2.15c

Means within each column followed by the same letter are not significantly different ($P \geq 0.05$).

3.3.3 F1 sterility and per generation rate of increase

The different combinations of treated and untreated *T. leucotreta* per cage had a significant impact on the mean number of fertile (= progeny of unsterile males) F1 male *T. leucotreta* ($\chi 2 = 59.29$; df = 4; $P < 0.05$) and the mean number of fertile F1 female *T. leucotreta* ($\chi 2 = 52.09$; df = 4; $P < 0.05$) emerging from the damaged fruit in the different cages (Table 3.3). Control cages yielded significantly more F1 males and F1 females than other cages receiving different combinations of treated and untreated *T. leucotreta*, except for cages receiving treatment UM×UF×TM×TF. Among the cages receiving different combinations of treated and untreated *T. leucotreta*, treatment UM×UF×TM×TF produced significantly higher numbers of F1 males and females than treatments TM×UF, UM×TF, and TM×TF. Except for treatment UM×UF×TM×TF, the other cage treatments with different

combinations of treated and untreated *T. leucotreta* exhibited a lower rate of reproductive increase than the control cages (Table 3.3). The control cages exhibited the highest per generation rate of reproductive increase, with a mean rate of increase exceeding 4 for both males (4.35×) and females (4.13×) from P1 to the F1 generation. Consequently, the mean per generation rate of reproductive increase for both males and females was 4.24×. No surviving progeny was produced from the TM × TF combination. Therefore, no per generation rate of increase could be determined (Table 3.3). However, treatment TM × UF had a lower mean number of fertile F1 males and females, as well as a lower per generation rate of reproductive increase from the P1 to the F1 generation. This treatment resulted in a mean rate of increase that was <1 for both males (0.58×) and females (0.57×). Consequently, the mean per generation rate of reproductive increase for both males and females was (0.57×), a value resulting in a slight decline from P1 to the F1 generation (Fig. 3.4).

Table 3.3: Effect of different combinations of treated and untreated *T. leucotreta* on the mean number of fertile F1 female and male moths emerging from fruit removed from the cages and the reproductive rate of increase for the P1-F1 generation.

Combinations	Mean ± SE fertile moths (progeny of non-treated males)		P1-F1 reproductive rate of increase	
	F_1 male	F_1 female	Male	Female
Control (UM × UF)	43.56 ± 14.40c	41.33 ± 15.32c	4.35×	4.13×
TM × UF	5.89 ± 2.37b	5.78 ± 2.67b	0.58×	0.57×
UM × TF	6.56 ± 2.31b	5.67 ± 2.23b	0.65×	0.56×
TM × TF	0.00 ± 0.00a	0.00 ± 0.00a	0.00×	0.00×
UM × UF × TM × TF	32.56 ± 10.81c	31.11 ± 11.57c	3.25×	3.11×

Means within each column followed by the same letter are not significantly different ($P \geq 0.05$).

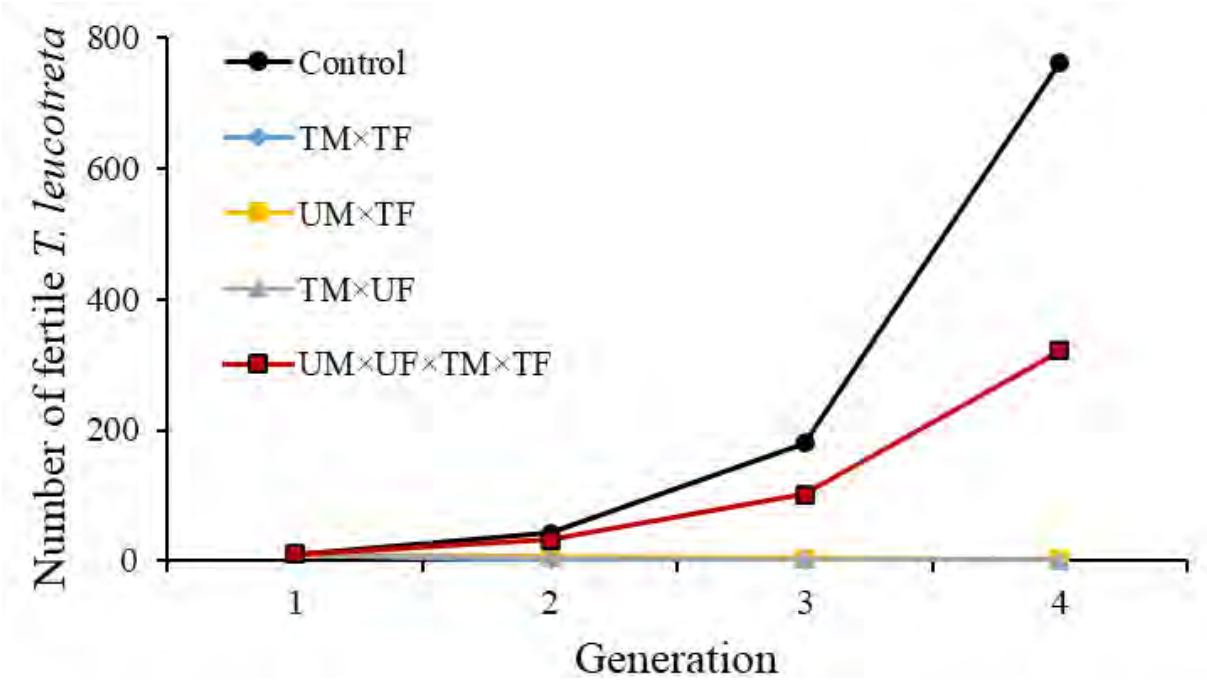

Fig. 3.4: Comparison of the projected rise in the count of fertile *T. leucotreta* between a control population and treatment populations exposed to releases of both treated and untreated *T. leucotreta*. In the control population, the initial generation began with 10 pairs of untreated *T. leucotreta* (ratio of 1:1), showing a reproductive rate of 4.24× per generation. Similarly, the treatment population TM×UF (= with a low per generation rate of increase) initiated generation 1 with the release of 10 pairs of both treated and untreated *T. leucotreta* at the start of each of the three generations (ratio of 1:1), resulting in a reduced reproductive rate of 0.57× per generation.

3.4 Discussion

The efficacy of insect management strategies like mating disruption, chemical sterilisation, and sterile insect techniques increasingly rely on the understanding of insect mating preferences (Parker & Mehta, 2007; Azrag et al., 2021; Pérez-Staples et al., 2021). Moreover, the success of SIT programmes hinges on the ability of treated male populations to compete with fertile wild males to copulate with the fertile wild females, thereby reducing the pest population and serving as an effective autocidal control (Parker & Mehta, 2007; Draz et al., 2016; Woods et al., 2016). In this study, we examined the mating capabilities of *T. leucotreta* under different combinations of treated and untreated moths and the impact on fruit damage and population growth. Our findings revealed that any cage treatment involving treated *T. leucotreta* resulted in reduced fruit damage and larval entries. Consequently, fewer

F1 adults emerged, except for the cage treatment with the UM×UF×TM×TF combination. This outcome may be attributed to the mating competitiveness of the released untreated *T. leucotreta*, which appeared to outcompete treated counterparts, resulting in more mating events and, thus, a greater transfer of fertile spermatophores between the untreated insects (Bloem et al., 2003; Calkins & Parker, 2005; Parker & Mehta, 2007; Woods et al., 2016). Consequently, this led to increased fruit damage, larval entries, and ultimately, a higher number of emerged F1 adults from the cages. Despite this, the per generation rate of increase over several generations indicated that the presence of sterile *T. leucotreta* in the UM×UF×TM×TF combination, even under the recommended minimum ratio of 10:1 (sterile: wild) could have a suppressant effect on the fertile wild population.

Additionally, the findings from the UM×UF×TM×TF combination, exhibiting higher levels of fruit damage, larval entries, and emerged F1 adults than other treatments apart from the UM×UF (control), could be attributed to factors other than just the sterilisation with the 180 Gy gamma radiation. These factors may include transportation under chilling conditions, known to impact the fitness of *T. leucotreta* (Nepgen et al., 2015). According to Nepgen et al. (2015), both males and females experienced decreased flight ability due to irradiation, wing injuries during transportation, chilling, and seasonal effects, particularly noticeable in summer relative to winter conditions, owing to the drastic temperature change from chilled to warmer environmental conditions upon release or during preparation for releases. This did greatly affect the spermatophore production and transfer between the treated and untreated *T. leucotreta*, but could also have compromised mobility, visual ability, and pheromone detection by treated male *T. leucotreta*, due to irradiation, thereby affecting transferal of spermatophores to the female *T. leucotreta* (Calkins & Parker, 2005). Similarly, Mutika et al. (2001) demonstrated that treated adult *Glossina pallidipes* (Austen) (Diptera: Glossinidae) males transferred significantly less sperm to females than their untreated male counterparts. This could be due to exposure of the adult *G. pallidipes* to chilling temperature affecting their copulation ability, hence low sperm transfer (Mutika et al., 2002).

The findings are congruent with those reported by Woods et al. (2016) on the Australian light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), indicating that releases of sterile males alone have the potential to enhance mating competitiveness of the treated moths as compared to the dual-sex moth releases. Van steenderen (2017) demonstrated that a higher suppressive effect is achieved in the dual-sex releases relative to male-only releases resulting in a reduction in fruit damage and larval entries, thereby aiding in *T. leucotreta* suppression. Similarly, Hight et al. (2005) reported that

dual-sex releases can be more effective than male-only releases in controlling *C. cactorum*. However, the findings by Moore et al. (2021) align with our results, demonstrating significantly fewer damaged fruit, larval entries, and F1 *T. leucotreta* adults in any cage treatment receiving either treated male or female *T. leucotreta* compared to the control (untreated males and females). Similarly, our findings align with those reported by Bloem et al. (1999) on *C. pomonella*, demonstrating a reduction in damaged fruit, larval entries, and emerged F1 adults in treatments receiving treated insects as compared to the control. However, these findings contrast with those of Marti & Carpenter (2009), who did not record any significant differences in the transfer of spermatophore or mating by untreated and treated males (sterilised at 200 Gy) of *C. cactorum*. Likewise, White et al. (1975) did not report any significant difference in sperm transfer between treated (300 Gy) and untreated *C. pomonella*, however, a delay in the transfer of spermatophore to the female's bursa copulatrix was reported in the study. Theoretically, this delay could reduce the efficacy of sterile sperm fertilising eggs if females are polyandrous (as *T. leucotreta* females are) because fertile/untreated males' sperm could potentially fertilise eggs sooner (White et al., 1975).

Various combinations of treated and untreated *T. leucotreta* significantly impacted the mean number of emerging F1 males and F1 females from the different cage treatments compared to the control cages, where a higher number of F1 males than F1 females were recorded. This can be attributed to the radiation dosage employed, as *T. leucotreta* males exhibit radio-tolerance, resulting in partially sterile *T. leucotreta* males (Marec et al., 2021). When these partially sterile males copulate with fertile/untreated females, the radiation-induced detrimental effects are inherited by the F1 generation, leading to reduced egg hatch rates and the resultant offspring being both fully sterile and predominantly male (Marec et al., 2021). The findings are consistent with those reported by Moore et al. (2021), indicating a decrease in the F1 adults from parental generation (P1) to filial generation (F1) across various combinations of treated and untreated *T. leucotreta*. Similarly, they align with the findings of Bloem et al. (2003), demonstrating the effects of F1 sterility resulting from irradiation of P1 males with specific radiation dosages to the F1 generation, where decreased F1 fecundity and fertility, increased F1 mortality during development, and a significant shift in the F1 sex ratio in favour of males were observed. Likewise, Carpenter et al. (2001) and Hight et al. (2005) reported similar results for *C. cactorum*, where inherited effects resulting from irradiation of P1 males and females were manifested in the F1 generation, leading to increased developmental time from oviposition to larval eclosion, elevated egg mortality, and increased mortality from neonate to adult stage. Comparable outcomes were also reported in studies by

Bloem et al. (1999, 2001) on *C. pomonella* and Cagnotti et al. (2016) on *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae).

The findings from this study suggest that employing various combinations of treated and untreated *T. leucotreta* adults can effectively mitigate fruit damage and reduce the number of emerging F1 offspring under laboratory conditions. These findings align with those reported by Hofmeyr et al. (2005). However, accurately predicting the impact of releasing treated and untreated *T. leucotreta* into a wild population requires assessing its efficacy over several generations (Hofmeyr et al., 2005). In the study, we simulated a typical scenario of a SIT programme, where treated and untreated *T. leucotreta* would be continuously released from the beginning of a season when the wild *T. leucotreta* population is at its lowest. A comparison of reproductive rates was conducted between two populations: one comprising untreated *T. leucotreta* males and females (control) and the other consisting of treated and untreated *T. leucotreta* (exposed to 180 Gy gamma radiation).

The initial control population consisted of 10 pairs of untreated male and female *T. leucotreta*, exhibiting an average reproductive rate of 4.24× per generation. Conversely, in the treated population, the first generation started with 10 pairs of treated and untreated *T. leucotreta* at the start of each of the three generations (at a 1:1 ratio), resulting in a reduced reproductive rate of 0.57× per generation for both male and female *T. leucotreta*. Based on our model, derived from our laboratory data, the population receiving treated and untreated *T. leucotreta* treatment experienced a decline, while the control population saw a growth of more than 4-fold per generation. This outcome is similar to the findings reported by Hofmeyr et al. (2005), which demonstrated that the population receiving treated *T. leucotreta* at an overflooding ratio of 10T:1U decreased in number, while the control population increased in number by more than 9-fold in four generations. This also aligns with Chapter 2 findings, see Result section “2.3.2 fecundity and fertility section”. The treatment combination TM × TF did not produce any surviving F1 progeny as the treated females are known to be radiosensitive, thereby resulting in completely sterile females (Bloem et al., 2003). However, contrary to the findings of Bloem et al. (2003), some percentage of fruit damage was reported in combinations involving treated males and females. This could be attributed to insufficient radiation dose enough to render the female moths fully sterile. This is against the principles of XSIT and needs to be rectified by increasing the radiation dose (180 Gy) for treating the moths, to ensure the females achieve complete sterility without affecting their mating competitiveness.

In summary, our findings demonstrated that untreated *T. leucotreta* males mated more frequently with untreated females compared to combinations involving treated and untreated *T. leucotreta* males and females. Consequently, this led to significantly greater differences in the percentage of damaged fruit, the mean number of larval entries, and F1 adult emergence per treatment. Although, the per generation rate of population growth was notably higher in both control cages and the treatment cages receiving dual-sex releases of both treated and untreated *T. leucotreta* males and females (UM×UF×TM×TF combination). Our study showed that any combination of treated and untreated moths can have a suppressant effect on *T. leucotreta*, as it provides the opportunity for some fertile-sterile pairings, rather than fertile-fertile pairings. Our findings support those of van Steenderen (2017), which suggested that dual-sex releases improve the efficacy of SIT. Dual-sex releases of sterile *T. leucotreta* have been conducted since the technique's inception in 2007, as the separation of sterile males and females is not practically feasible (XSIT, 2018). Nevertheless, the findings from this study were conducted under a controlled, confined environment, and the results warrant further investigation under semi-field and field conditions, since the primary goal of any SIT programme is for treated *T. leucotreta* to mate with wild fertile counterparts and increasing the over-flooding ratio of treated to wild insects enhances the likelihood of this occurring (Barnes et al., 2015). If wild males mate as readily with treated *T. leucotreta* as with wild females, treated females will serve as a positive sperm sink. Our study's findings support the ongoing improvement of the efficacy and effectiveness of the SIT programme as a strategy for controlling and managing *T. leucotreta* in South Africa.

CHAPTER 4

Effects of different release ratios and combinations of treated and untreated male and female *T. leucotreta* on fruit damage and population growth in a field cage study

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4.1 Introduction

Thaumatotibia leucotreta is a key phytosanitary pest attacking a wide range of cultivated and wild host plants in sub-Saharan Africa (EFSA, 2024). Amongst the cultivated fruit, citrus is the most preferred, particularly Navel oranges (Stofberg, 1954; Moore & Manrakhan, 2022; EFSA, 2024). South Africa is the second biggest exporter of citrus and must effectively control *T. leucotreta* due to the stringent phytosanitary regulations imposed by export markets (Moore, 2022; Moore & Manrakhan, 2022). *Thaumatotibia leucotreta* often causes premature fruit drop before harvest, and severe infestations can result in significant crop losses and potential rejection at packing houses (Hofmeyr et al., 2005, 2015; EFSA, 2024). To suppress *T. leucotreta*, area-wide integrated pest management programmes employ a combination of techniques (Moore, 2021), one of which is the sterile insect technique (SIT), widely regarded as a reliable and environmentally benign method for controlling *T. leucotreta* (Barnes et al., 2015; Hofmeyr et al., 2015).

The application of SIT involves releasing a large number of sterile insects into the target area at a certain sterile-to-wild ratio, which increases the likelihood of matings between sterile males and wild females (Knipling, 1955; Enkerlin, 2005; Mounika et al., 2022). These matings result in the production of non-viable eggs, thereby inducing sterility in the wild population (Mounika et al., 2022). The effectiveness of sterility induction under normal control operations improves when sterile males successfully outcompete wild males after multiple releases, ultimately slowing the intrinsic population growth rate (Mastrangelo et al., 2018). The release ratios and the irradiation dose required to induce sterility vary between different insects, with Lepidoptera and Coleoptera requiring higher doses (Klassen, 2005; Mounika et al., 2022). Accurately estimating the sterile-to-wild release ratio is imperative for determining the number of sterile insects needed and the success of a SIT programme (Rendón et al., 2004; Flores et al., 2017). Although a higher release ratio may be achieved, the percentage of sterility induced may still be low, indicating that the mating competitiveness of sterile insects is a critical factor (Rendón et al., 2004; Shelly & McInnis, 2016; Mounika et al., 2022). For instance, Rendón et al. (2004) reported that dual-sex releases with ratios above 1000:1 induced only 12% sterility, whereas single-sex releases with 100:1 release ratios achieved a sterility index of 70% in *C. capitata*. Likewise, Shelly and McInnis (2016) reported that *Z. cucurbitae* populations were reduced by more than 99% at a release ratio of 50:1 compared to *C. capitata* of between 50-93% at a release ratio of 700:1 to 3160:1 in Nicaragua, whereas 100:1 to 400:1 achieved 80% population reduction.

Hight et al. (2005) determined that a release ratio of 5:1 could effectively control *C. cactorum*.

Similarly, in *T. leucotreta*, Hofmeyr et al. (2005) found in a field cage study that a release ratio of 10:1 was effective in inducing sterility within the fertile population, leading to pest suppression. As a result, this release ratio was adopted as the minimum benchmark for the *T. leucotreta* SIT programme in South Africa since the commercial inception of the technique in 2007. Likewise, my findings in Chapter 2 indicated that release ratios above 40:1 can be more effective than 10:1 in suppressing *T. leucotreta*. Additionally, some field trials have suggested that higher release ratios might be more effective than the 10:1 release ratio (Hofmeyr & Hofmeyr, 2009, 2010; Moore, 2011), highlighting the need to evaluate the efficacy of release ratios greater than 10:1 in a field cage study. In a *T. leucotreta* SIT programme, both sterile males and females are released, since sorting the moths is impractical, due to the large number of sterile insects required (XSIT, 2018). Moore et al. (2021) recorded some fruit damage from certain pairings of sterile and fertile males and female *T. leucotreta*. Similarly, this was also demonstrated in my findings in Chapter 3. However, van Steenderen (2017) reported that sterile females act as a positive sperm sink for wild males rather than a negative one. Therefore, the aim of this chapter was to assess the effects of different release ratios higher than 10:1 and different combinations of sterile and fertile *T. leucotreta* males and females on fruit damage and population growth rate in a field cage study.

4.2 Materials and Methods

4.2.1 Study site

The experiments took place in a 10 ha commercial, organically managed, 9 year old Nova mandarin *Citrus reticulata* (Blanco) (Sapindales: Rutaceae), orchard, in the Sundays River Valley, Eastern Cape Province, South Africa (Fig. 4.1).

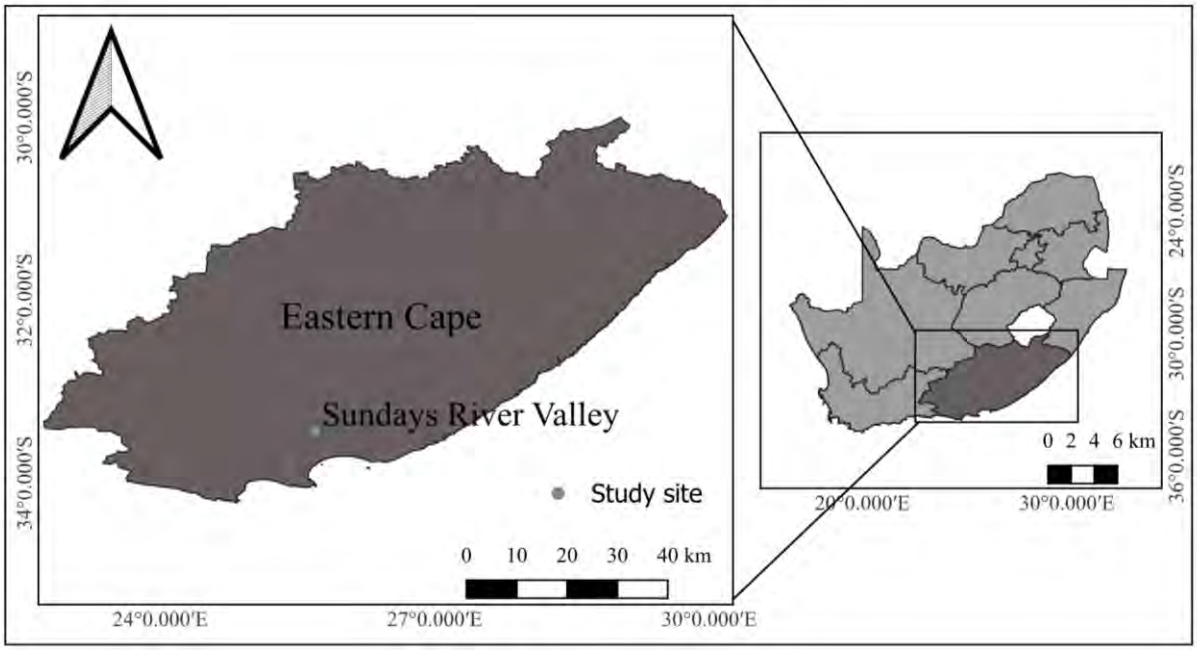

Fig. 4.1: Location of the study site in the Sundays River Valley.

4.2.2 Test insects

Adult sterile and fertile *T. leucotreta* were sourced from XSIT, Citrusdal, South Africa. To render them sterile, adult moths were exposed to a dose of 180 Gy of gamma irradiation. Thereafter, the moths were transported to Rhodes University, Makhanda, South Africa (~840 km, 13-14 h) in a polystyrene cooler box with dry ice bricks, maintaining a temperature of 4-6°C. This controlled temperature was crucial in minimising the moths' activity and preventing mating while in transit (Nepgen et al., 2015). The moths were approximately 48 h old upon arrival and were kept in a refrigerator at 5°C and immediately sorted by sex using a dissection microscope (Zeiss Microscopy, South Africa) at a magnification of 40× to prevent any unintended mating. Distinguishing characteristics included the presence of black anal tufts on the hind tibiae in males, which are absent in females (Gilligan et al., 2011).

4.2.3 Release of sterile *T. leucotreta* into the field cages

The field cage studies were conducted as per the methodology outlined by Hofmeyr et al. (2005). A total of 15 nylon-mesh field cages (measuring 3 m by 3 m by 3 m) with wooden support frames were used to enclose individual Nova mandarin trees (average tree height 2.3 m) (Fig 4.2). The mesh size of the cages was 125 µm, sourced from Mesh-Tech (Pty) Ltd., Fourways, South Africa. The trees were caged for 6 weeks after moth release.

Fig. 4.2: A Nova mandarin tree enclosed in a mesh cage.

Before the release of moths into the cages, the adult sterile and fertile moths were sorted into different ratios, as indicated in Table 4.1. This grouping was done one day before the release, with male and female *T. leucotreta* being released on opposite sides of each tree. The selection of treatments within the cages was randomised, with each treatment being replicated three times. Throughout the trial period, the insects were allowed to mate and lay eggs without any disturbance. Regular inspections of the cages were carried out every two days, during which any dropped fruit were collected and transferred to the laboratory. The fruit were thoroughly examined for damage (presence of brown larval penetration holes/frass spots), and the number of larval entries per fruit was carefully recorded for each replicate of the respective treatment. The experiment was repeated three times.

Table 4.1: Randomly assigned treatments in 15 field cages to examine the effect of release ratios of treated (T) to untreated (U) *T. leucotreta* on the incidence of fruit damage and the competitiveness of sterile males.

Treatment	Release ratio T: U	Number of treated *T. leucotreta*		Number of untreated *T. leucotreta*		
		T male	T female	U male	U female	Total moths
A	0:1(Control)	0	0	10	10	20
B	10:1	100	100	10	10	220
C	20:1	200	200	10	10	420
D	40:1	400	400	10	10	820
E	60:1	600	600	10	10	1220

Similarly, the field cage studies to determine the effects of different combinations of treated and untreated male and female *T. leucotreta* were conducted as per the aforementioned methodology. Different combinations of treated and untreated male and female *T. leucotreta* were used as per Table 4.2. The experiment was repeated three times.

Table 4.2: Randomly assigned treatments in 15 field cages to examine the effect of different combinations of treated and untreated *T. leucotreta* on fruit damage and population growth.

Treatment	**1**	**2**	**3**	**4**	**5**
Combination	UM×UF	TM×UF	UM×TF	TM×TF	UM×UF×TM×TF
Untreated	30 30	30	30	30 30	
Treated		30	30	30 30	30 30

UM- untreated male, UF- untreated female, TM- treated males, TF- treated females. A ratio of 1:1 was used between treated and untreated *T. leucotreta* males and females.

4.2.4 Statistical analysis

Data collected from both experiments were checked for homogeneity of variance (F test, Levene's test) and the residual deviations for non-normality were determined using the

Shapiro Wilk test, revealing that the data were not normally distributed (Shapiro & Wilk, 1965). The data were analysed using a negative binomial generalised linear model analysis as an extension of the Poisson distribution to allow for count data with a significant proportion of zero values, with different ratios as the sources of variation, as recommended by O'Hara and Kotze (2010). Analysis of deviance (log-likelihood ratio statistic) was used to assess the goodness of fit of the Poisson regression model, which has a distribution similar to that of chi-squared ($\chi 2$). The dependent variables used in the statistical model included the number of larval entries and the number of damaged fruit from the different treatments. Differences among treatment means were separated using the Tukey-Kramer statistic ($P \leq 0.05$) for multiple comparisons when the statistical model indicated significant treatment effects. All analyses were performed in R. 4.2.2 (R Core Team, 2023).

4.3 Results

The different release ratios significantly affected the mean number of damaged fruit ($\chi 2 = 10.42$; df = 4; $P = 0.03$) and the mean number of larval entries ($\chi 2 = 9.36$; df = 4; $P < 0.05$) (Table 4.3). The mean number of damaged fruit at the release ratio of 20:1 was significantly higher than at all other release ratios. Similarly, a significantly higher mean number of larval entries was recorded at the 20:1 release ratio compared to the 40:1 ratio. Interestingly, no clear trend emerged between increasing release ratios and the level of fruit damage caused.

Table 4.3: Effect of different release ratios on the mean number of damaged fruit and larval entries per treatment.

Release ratio	Mean number of damaged fruits ± SE per cage	Mean number of larval entries ± SE per cage
0:1 (Control)	0.57 ± 0.25a	1.87 ± 0.92ab
10:1	0.43 ± 0.24a	1.43 ± 0.89ab
20:1	1.29 ± 0.48b	2.86 ± 1.02b
40:1	0.05 ± 0.05a	0.05 ± 0.05a
60:1	0.62 ± 0.29a	1.38 ± 0.78ab

Means within each column followed by the same letter are not significantly different ($P \geq 0.05$).

The different combinations of treated and untreated male and female *T. leucotreta* did not significantly affect the mean number of damaged fruit ($\chi 2 = 17.45$; df = 4; $P > 0.05$) and the mean number of larval entries ($\chi 2 = 14.38$; df = 4; $P > 0.05$) (Table 4.4).

Table 4.4: Effect of different combinations of treated and untreated male and female *T. leucotreta* on mean number of damaged fruit and larval entries per treatment.

Combination	Mean number of damaged fruits ± SE per cage	Mean number of larval entries ± SE per cage
UM×UF (Control)	0.57 ± 0.29a	1.52 ± 0.82a
TM×UF	0.76 ± 0.28a	1.81 ± 0.66a
UM×TF	0.19 ± 0.11a	0.67 ± 0.42a
TM×TF	0.00 ± 0.00a	0.00 ± 0.00a
UM×UF×TM×TF	0.81 ± 0.34a	1.48 ± 0.65a

Means within each column followed by the same letter are not significantly different ($P \geq 0.05$).

4.4 Discussion

Since SIT relies on the mass release of sterile insects, it is important to accurately estimate the release ratio to ensure the success of any SIT programme (Shelly & McInnis, 2016; Mastrangelo et al., 2018). Additionally, the optimum sterilisation dose used is also important as it influences the mating competitiveness of the released insects. In the case of dual-sex releases, such as with *T. leucotreta*, the minimum dose used must ensure complete sterility in females and partial sterility in males, to maintain their mating vigour with wild females (Mastrangelo et al., 2018; Mounika et al., 2022). However, the processes of mass-rearing, sterilisation, and inundative release can introduce changes that may negatively impact the sexual behaviour of both sterile and fertile insects (Orozco et al., 2007), potentially influencing their mating behaviour (Aigbedion-Atalor et al., 2024a, b).

In the review of the release ratio studies by Hofmeyr et al. (2005), this study evaluated the effectiveness of higher release ratios than 10:1, on fruit damage and population growth in field cage studies. Our results showed that, except for the 20:1 release ratio, there was no significant difference between the control treatment and the other treatments. There was also no clear trend in the mean number of damaged fruit and larval entries as the release ratio increased. This finding contrasts with the results of Hofmeyr et al. (2005) and the

laboratory study (Chapter 2), which indicated a negative correlation between the mean number of damaged fruit and larval entries as the ratio of sterile insects increased. Similar outcomes were reported by previous studies, including Bloem et al. (1999) on *C. pomonella*, Hight et al. (2005) on *C. cactorum*, Flores et al. (2014) on *A. ludens*, and Shelly & McInnis (2016) on *B. dorsalis*, *C. capitata* and *Z. cucurbitae.* Additionally, the different combinations of sterile and fertile male and female *T. leucotreta* in the field cages did not significantly affect the mean number of damaged fruit and larval entries. This is in contrast to the laboratory results presented in Chapter 3 and the findings of Moore et al. (2021), which showed some fruit infestation and larval entries resulting from certain pairings of treated and untreated males and females of *T. leucotreta*.

These negative results could have stemmed from various factors in the field affecting moth performance, which should be addressed in future research. Factors such as microclimatic conditions, including temperature and rainfall might have impacted the moth's fitness. For example, sterile moths do not fly at temperatures below 16°C which might lead to a lack of mating opportunities (Stotter & Terblanche, 2009). Thus, monitoring climatic variables using data loggers could help track fluctuations in cage conditions and also aid in determining the appropriate time to conduct the releases. Additionally, *T. leucotreta* attacks a wide range of citrus varieties (Newton, 1998), excluding lemons and limes (Moore et al., 2015). Therefore, using a citrus variety that *T. leucotreta* strongly prefers, such as Navel oranges, and replicating the study across several orchards would provide a comprehensive representation of different orchards across the citrus-growing areas, rather than limiting it to just one orchard. The cultivar used, Nova mandarin, is known to be susceptible to *T. leucotreta* attack, as this variety was used in field trials to validate the *T. leucotreta* systems approach for exports of citrus from South Africa to the European Union (Hattingh et al., 2020), but not to the same extent as Navel oranges and possibly not sufficiently so to detect notable differences between treatments. Conducting two or more releases per trial, rather than a single release, might have mitigated any potential issues with moth quality, which could have improved the outcome, given that the male moths have an average lifespan of only 3.97 days in summer and 6.88 days in winter (Nepgen et al., 2015).

The quality of the sterile moths is crucial, and they should be released within 48 h after irradiation to ensure maximum longevity and minimise quality deterioration (Hofmeyr et al., 2019). Hofmeyr et al. (2005) conducted the study in Citrusdal region which is closer to the XSIT facility and sterile moths do not require long distance transportation, aiding in

release of younger and high-quality moths. However, in our study due to the time required for moth transportation and sorting, this process extended beyond 72 h, which could have potentially impacted the moth quality. Therefore, ensuring that moths are released within 48 h is recommended for better performance in the field. Additionally, the number of sterile and fertile *T. leucotreta* released in the field cages might have been low, reducing the chances of the sterile and fertile male and female *T. leucotreta* matings. Therefore, it would be prudent to increase the numbers released while still maintaining the correct release ratios. In conclusion, addressing these factors might potentially improve the outcomes of these field cage trials.

CHAPTER 5

Suitability of false codling moth eggs from different sterile to fertile moth ratios in the sterile insect technique programme, to parasitism by *Trichogrammatoidea cryptophlebiae*

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5.1 Introduction

Thaumatotibia leucotreta is endemic to SSA and exhibits an extensive host range in southern Africa, including citrus, particularly Navel oranges (Stofberg, 1954; Economides, 1979; Yahia et al., 2011; Maniania et al., 2017; Moore, 2021; Moore & Manrakhan, 2022). *Thaumatotibia leucotreta* exhibits a multivoltine life cycle with four to six overlapping generations per annum in South Africa (Stofberg, 1954). Females typically deposit about 100-250 eggs on fruit, dependent mainly on temperature, relative humidity, and food quality (Daiber, 1980; Adom et al., 2021). After hatching, the newly emerged neonates penetrate the fruit, where they undergo five larval instars. Upon completing their development, larvae exit the fruit and create cocoons, either within the topsoil or leaf litter (Stofberg, 1954; Georgala, 1969; Moore, 2022). *Thaumatotibia leucotreta* commonly leads to premature fruit drop before harvest (Georgala, 1969). Severe infestations can have significant impacts, causing notable crop losses and potential rejections at packing houses (Hofmeyr et al., 2005). However, over the last two decades, control measures in the field have improved to the extent that severe levels of infestation are now rare (Moore et al., 2016, 2017; Moore, 2022).

Thaumatotibia leucotreta has been declared a key phytosanitary pest in many export regions, due to its endemism in the sub-Saharan region (Hattingh et al., 2020) and zero-tolerance policy in certain export markets. The growing global demand for fresh citrus fruit has heightened export market concerns about the potential introduction of this pest (Yahia et al., 2011; Moore, 2021). This is despite *T. leucotreta* being a poor disperser and coloniser (Moore et al., 2024), having established in only one region, outside of its endemic range, Israel (Wysoki, 1986), despite large volumes of fresh produce being shipped intercontinentally for many decades. Given that South Africa is the second-largest exporter of citrus, with approximately 75% of its citrus crop destined for export (CGA, 2023), it is crucial to ensure that exemplary control measures are undertaken before shipping (Moore, 2021). Consequently, the demand for high-quality fruit in the export market has translated into a need for new, efficient, and effective IPM strategies. These IPM approaches employ a harmonious combination of both chemical and ecologically sound techniques to manage and suppress pest populations to keep them below economic injury thresholds and to ensure they remain at acceptable phytosanitary levels (Malan et al., 2018). The use of several different control strategies together helps compensate for the drawbacks associated with any one strategy used alone, with monitoring being a focal point in helping to decide which control method to use (Urquhart, 1999; Carpenter, 2000; Malan et al., 2018).

Among the available methods of *T. leucotreta* control, not one is sufficiently effective to be used as a stand-alone tactic when pest pressure is high. Therefore, it is imperative that *T. leucotreta* control be based on a multidisciplinary approach, focusing on pest suppression from the start of the season and curbing population increase during the season (Moore & Hattingh, 2012; Moore, 2022). Thus, the demand for a long-term solution for the management of *T. leucotreta* necessitated the citrus industry to initiate a sterile insect technique research programme in Citrusdal, Western Cape Province, South Africa in 2002 (Barnes et al., 2015). This was conducted in several phases, where Bloem et al. (2003) determined that a radiation dose of 150 Gy completely sterilised female *T. leucotreta*, while male *T. leucotreta* were partially sterilised. This aids the males in maintaining their competitiveness in the field for pairing with fertile females, against their fertile wild male counterparts. However, full sterility is inherited by the F1 generation of an irradiated male-wild female pairing. Additionally, Hofmeyr et al. (2005) determined an ideal release ratio of 10:1, which is still in use. This was followed by the commercialisation of the technique in 2007 (Hofmeyr et al., 2016) as an AW-IPM strategy, in which other orchard-specific techniques can be incorporated, all aiding in the suppression of *T. leucotreta* populations in citrus orchards (Moore & Hattingh, 2012; Malan et al., 2018). The technique is host-specific and environmentally benign to natural enemies; therefore, it is compatible with the application of augmentative biological control (Mannion et al., 1994; Moore, 2002; Bloem et al., 2007; Horrocks et al., 2020).

Augmentative releases of egg parasitoids and sterile insects as biological control agents have been shown to significantly reduce pest damage to citrus (Newton & Odendaal, 1990; Bloem et al., 1998; Carpenter et al., 2004; Kaspi & Parrella, 2006; Bloem et al., 2007; Horrocks et al., 2020). Despite little empirical supporting data, some models predict mutual complementation (Barclay, 1987) or a mutual synergistic suppressive effect (Knipling, 1998) when the two techniques are used together. The mobility and enhanced host-searching capacity of egg parasitoids and sterile insects improve the efficacy of locating their target hosts in their natural habitats. Egg parasitoids and sterile insects can reach areas that other techniques, such as chemical sprays, are unable to reach (Pérez-Staples et al., 2021) and are complementary because they act on two different stages of the pest life cycle. As such more effort needs to be directed toward the control of the egg stage before the emergence of the larvae, which is the developmental stage of *T. leucotreta* responsible for crop damage (Newton & Odendaal, 1990; Moore, 2002; Schäfer & Herz, 2020; Masry & El-Wakeil, 2020). *Trichogrammatoidea cryptophlebiae* is a naturally occurring *T. leucotreta* egg

parasitoid found in citrus orchards (Newton & Odendaal, 1990; Moore, 2002). However, they can be commercially purchased to boost their numbers in citrus orchards, thereby improving their efficacy. This parasitoid has been shown to reduce *T. leucotreta* infestation of fruit in citrus orchards by up to 60% under optimum conditions (Newton & Odendaal, 1990; Moore, 2002). Commercial mass-rearing and augmentation of *T. leucotreta* egg parasitoids in South Africa have been conducted since 1982, when it began in the Western Cape Province, achieving a decrease in infestation by 20% against *T. leucotreta* (Schwartz et al., 1982). For a long time, mass-rearing and production of egg parasitoids was conducted by Cederberg Insectary in Citrusdal in the Western Cape Province and at Zebediela Estate in Limpopo Province (Moore et al., 2014). Currently, there are two insectaries commercially rearing the parasitoid, Vitalbugs in Limpopo Province, and River Bioscience in the Eastern Cape Province (Moore, 2021).

These insectaries may not be sufficient to supply all citrus orchards in the country. However, conservation of naturally occurring parasitoids is also feasible, and with SIT being practiced in major citrus-producing areas across the country, these two techniques could be compatible. This combination could enhance the efficacy of the parasitoid in areas where it is augmented and could aid in the proliferation of egg parasitoids in areas where augmentative release is not possible. In a similar situation in the control of codling moth, *C. pomonella*, the combined release of sterile insects and *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae) provided improved control (Nagy, 1973). Bloem et al. (1998) showed that an additive suppressive effect could be achieved when sterile male *T. leucotreta* are released at a 10:1 (sterile to wild) overflooding ratio, together with *Trichogramma platneri* (Nagarkatti) (Hymenoptera: Trichogrammatidae) in field-cages, when compared with cages containing wild moths that received sterile moths or parasitoids only. However, achieving this overflooding ratio in a commercial field may be more challenging than in an insulated and protected cage environment. Nevertheless, some field trials using *T. leucotreta* SIT programmes have indicated that higher ratios may be more efficacious than achieved by 10:1 (Hofmeyr & Hofmeyr, 2009, 2010; Moore, 2011). Similarly, the findings in Chapter 2 revealed that any ratio from 40:1 can be more effective against *T. leucotreta*. Carpenter et al. (2004) determined the suitability of the *T. leucotreta* eggs from a ratio of 10:1 to egg parasitoids. However, ratios higher than 10:1 are often achieved under field conditions, necessitating the need to determine the acceptability and suitability of *T. leucotreta* eggs to egg parasitoids, from different sterile to fertile moth ratios, higher than 10:1.

Therefore, this study investigated the parasitism levels of *T. leucotreta* eggs as affected by different sterile to fertile *T. leucotreta* ratios and egg ages. In particular, the study investigated (i) the effect of different *T. leucotreta* egg ages from different sterile to fertile ratios higher than 10:1 on parasitism by the egg parasitoid, and (ii) the effect of different *T. leucotreta* egg ages from different sterile to fertile ratios on the fitness of emerging egg parasitoids. The results obtained are discussed in the context of improving *T. leucotreta* pest suppression through the combined release of egg parasitoids and sterile *T. leucotreta* in an AW-IPM programme in citrus orchards.

5.2 Materials and Methods

5.2.1 Test insects

5.2.1.1 Sterile and fertile *T. leucotreta*

Adult sterile and fertile *T. leucotreta* were obtained from XSIT, Citrusdal, South Africa. Adult moths were sterilised by exposure to 180 Gy of gamma irradiation at XSIT before being transported by road to Rhodes University, Makhanda, South Africa (~ 840 km, 13-14 h) in a cooler box containing dry ice bricks (4-6°C). This low temperature minimised insect movement, preventing them from mating while in transit (Nepgen et al., 2015). On arrival, the moths were approximately 48 h old. The male and female moths were immediately sexed and separated to prevent mating. Adult males were distinguished from females by the presence of black tufts of fine hairs in the anal region and hind tibiae, which are absent in females (Gilligan et al., 2011).

5.2.1.2 Parasitoids

Egg parasitoids (*T. cryptophlebiae*) were provided by River Bioscience (Pty) Ltd. (Gqeberha, South Africa). The facility uses surplus unrequired *T. leucotreta* eggs from XSIT, which serve as a suitable host material for the production of egg parasitoids. *Thaumatotibia leucotreta* eggs laid on wax egg sheets are sorted and exposed to egg parasitoids for 24 h to ensure the continuity of the colony. Eight parasitised wax egg sheets were transported to Rhodes University (~ 125 km, 2 h) in a cooler box with ice blocks. On arrival, they were immediately placed in a controlled environment (CE) room at 26 ± 1°C, a 14:10 (L:D) h photoperiod, and 65 ± 5% relative humidity (RH) for 48 h. Subsequently, they were allowed to mate for 24 h, after which they were sexed and used in subsequent experiments; approximately 50 female egg parasitoids were exposed to *T. leucotreta* eggs at different ratios

(Table 5.1). A fresh batch of egg parasitoids was supplied for each repetition of the experiment when needed.

5.2.2 Wax egg sheet preparation

The laboratory trials were conducted as described by Carpenter et al. (2004). Sterilised *T. leucotreta* adults were sorted according to sex. Sterile and fertile *T. leucotreta* were placed inside mesh domes (each 30 cm in diameter by 15 cm height) that were placed on top of wax oviposition sheets (32 cm by 15 cm) placed on top of a Styrofoam board in a CE room. The CE room conditions were maintained at 26 ± 1°C, a 14:10 (L:D) h photoperiod, and 65 ± 5% relative humidity (RH). Insect pins were used to secure the domes on top of wax egg sheets. Three replicates were set up for the different ratios of sterile to fertile moths: 0:1 (control), 10:1, 20:1, 40:1, and 60:1. The number of *T. leucotreta* used per treatment was as per Table 4.1. *Thaumatotibia leucotreta* were allowed to mate and oviposit on wax egg sheets under the aforementioned conditions for four days. The first wax egg sheet was collected after 12 h and disposed of to ensure that the eggs used in subsequent experiments came from matings with different sterile to fertile *T. leucotreta* ratios. Thereafter, three more wax egg sheets were collected at 24 h intervals. Newly laid (24 h), 48 and 72 h old eggs were evaluated. Wax egg sheets containing 200 *T. leucotreta* eggs from each ratio treatment were placed inside three meshed plastic containers (5 cm diameter by 3 cm height). Wax egg sheets collected on days 1 and 2 were incubated at 26 ± 1°C, a 14:10 (L:D) h photoperiod, and 65 ± 5% RH to allow for egg development (Carpenter et al., 2004). Thereafter, wax egg sheets, together with those collected on the third day, were exposed to egg parasitoids.

5.2.3 Exposure to egg parasitoids

Parasitised *T. leucotreta* wax egg sheets from River Bioscience (Pty) Ltd. were placed in large glass containers and kept at 26 ± 1°C, a 14:10 (L:D) h photoperiod, and 65 ± 5% RH for 12 h before the emergence of egg parasitoids. To ensure that only egg parasitoids that emerged within 24 h were used in the trial, adult egg parasitoids were collected from the container every 12 h and transferred to plastic Petri dishes. A mixture of honey and water (2:1) was used as food for egg parasitoids. To ensure mating success, egg parasitoids were kept together for 24 h. Thereafter, using a fine camel hair paintbrush, 50 female egg parasitoids were transferred to each plastic container, with approximately 200 *T. leucotreta* eggs on the wax egg sheet, and were allowed 24 h to parasitise *T. leucotreta* eggs, after which

they were removed. The wax egg sheets were then incubated under the aforementioned conditions for seven days to enable the completion of parasitoid development. After the emergence of egg parasitoids, data were collected on the number of parasitised eggs, the number of parasitised eggs that produced two or more egg parasitoids (superparasitised), the number and sex of the emerging wasps, the number of egg parasitoids that died before emergence for each treatment, and host egg age. The experiment was conducted three times, requiring a total of 15 fertile and 390 sterile males and 15 fertile and 390 sterile females, each time.

Table 5.1: Experiment design: egg parasitoids were randomly assigned to three plastic containers per treatment containing 200 *T. leucotreta* eggs of different ages laid from different ratios, to examine the acceptability and suitability of these eggs to parasitism by egg parasitoids.

	Ratio	Number of sterile *T. leucotreta*		Number of fertile *T. leucotreta*	
Treatment	S: F	S male	S female	F male	F female
A	0:1	0	0	1	1
B	10:1	10	10	1	1
C	20:1	20	20	1	1
D	40:1	40	40	1	1
E	60:1	60	60	1	1

S = sterile moths; F = fertile moths. Three replicates per treatment were used.

5.2.4 Parasitoid quality

Parasitised *T. leucotreta* wax egg sheets were kept in a CE room under the aforementioned conditions until wasp emergence. Upon emergence, the quality of the egg parasitoids was assessed using a flight test. From the time of emergence of the first egg parasitoids, an interval of 48 h was given to ensure the complete emergence of all the egg parasitoids from the host eggs. The flight chamber test was conducted using a protocol and flight chamber setup originally proposed by Dutton & Bigler (1995) and modified by Prezotti et al. (2002). The flight chamber was a PVC cylinder (11 cm in diameter and 18 cm high),

where the top of the cylinder was covered with a clear Petri dish (13 cm diameter) and sprayed with entomological glue (composed of polybutene and synthetic silica) (EntomoAlex-gr, Italy) to trap flying parasitoids ('flyers'). The interior of the cylinder was painted black, and the bottom was sealed with a flexible black plastic sheet to attract insects toward the light at the top of the flight chamber. Twenty-four hours before the start of the experiment, entomological glue was spread on the walls of the cylinder (3.5 cm from the bottom) to serve as a trap for walkers. The number of egg parasitoids trapped in the adhesive ring ('walkers'), the Petri dish ('flyers'), and the number of deformed individuals were recorded. The flyers were sexed to determine the sex ratio for each treatment. The experiment was conducted thrice.

5.2.5 Statistical analysis

Data collected from the experiment were checked for homogeneity of variance (F test, Levene's test) and normal distribution of residuals (Shapiro-Wilk test) (Shapiro & Wilk, 1965). As parametric assumptions were not met, non-parametric tests were used. The data were analysed using a negative binomial generalised linear model analysis as an extension of the Poisson distribution to allow for count data with a significant proportion of zero values, with ratios and egg age as the sources of variation, as recommended by O'Hara and Kotze (2010). Analysis of deviance (log-likelihood ratio statistic) was used to assess the goodness of fit of the Poisson regression model, which has a distribution similar to that of chi-squared (χ^2). The dependent variables used in the statistical model included the number of parasitised eggs, the number of super parasitised eggs, the number of emerged egg parasitoids, the sex ratio of emerged egg parasitoids, the number of flying male and female egg parasitoids, and the number of walking and flying egg parasitoids. Tukey's post-hoc comparisons were conducted at $P < 0.05$, where the statistical model indicated significant treatment effects and significant interactions. All statistical analyses were conducted using the R software version 4.3.1 (R Core Team, 2023).

5.3 Results

5.3.1 Rate of parasitism

The proportion of parasitised *T. leucotreta* eggs was significantly influenced by the different ratios ($\chi^2 = 64.15$; $df = 4$; $P < 0.05$) (Fig 5.1A). Similarly, egg age had a significant effect on the proportion of parasitised *T. leucotreta* eggs, irrespective of the ratio ($\chi^2 = 41.53$;

df = 2; $P < 0.05$). There was a significant difference in the proportion of parasitised *T. leucotreta* eggs between the control (0:1) and 40:1 as well as control and 60:1 at egg age 48 h (Fig 5.1A). However, no significant differences were recorded between 0:1 and other ratios at egg ages 24 and 72 h. At the ratio 0:1, the proportion of parasitised *T. leucotreta* eggs at the egg age of 72 h was significantly less than *T. leucotreta* eggs parasitised at the ages of 24 and 48 h. This trend was similar for ratios 20:1, 40:1, and 60:1. Conversely, the proportion of *T. leucotreta* eggs parasitised at a ratio of 10:1 at egg ages 48 and 72 h was significantly different from *T. leucotreta* eggs parasitised at the egg age of 24 h. No significant interaction was recorded between the different ratios and egg ages ($\chi^2 = 11.61$; *df* = 8; *P* = 0.17).

The proportion of superparasitised *T. leucotreta* eggs was found to be significantly affected across the different ratios ($\chi^2 = 12.21$; *df* = 4; $P < 0.05$) (Fig. 5.1B). Similarly, different egg ages also had a significant effect on the proportion of superparasitised eggs across the ratios ($\chi^2 = 51.56$; *df* = 2; $P < 0.05$). The proportion of superparasitised *T. leucotreta* eggs was significantly higher between ratio 0:1 and other release ratios, at the egg age of 24 h (Fig. 5.1B). However, there was no significant difference in superparasitised eggs between 0:1 and other ratios at the egg ages of 48 and 72 h. No significant interaction was recorded between the different ratios and the egg ages ($\chi^2 = 11.91$; *df* = 8; *P* = 0.16).

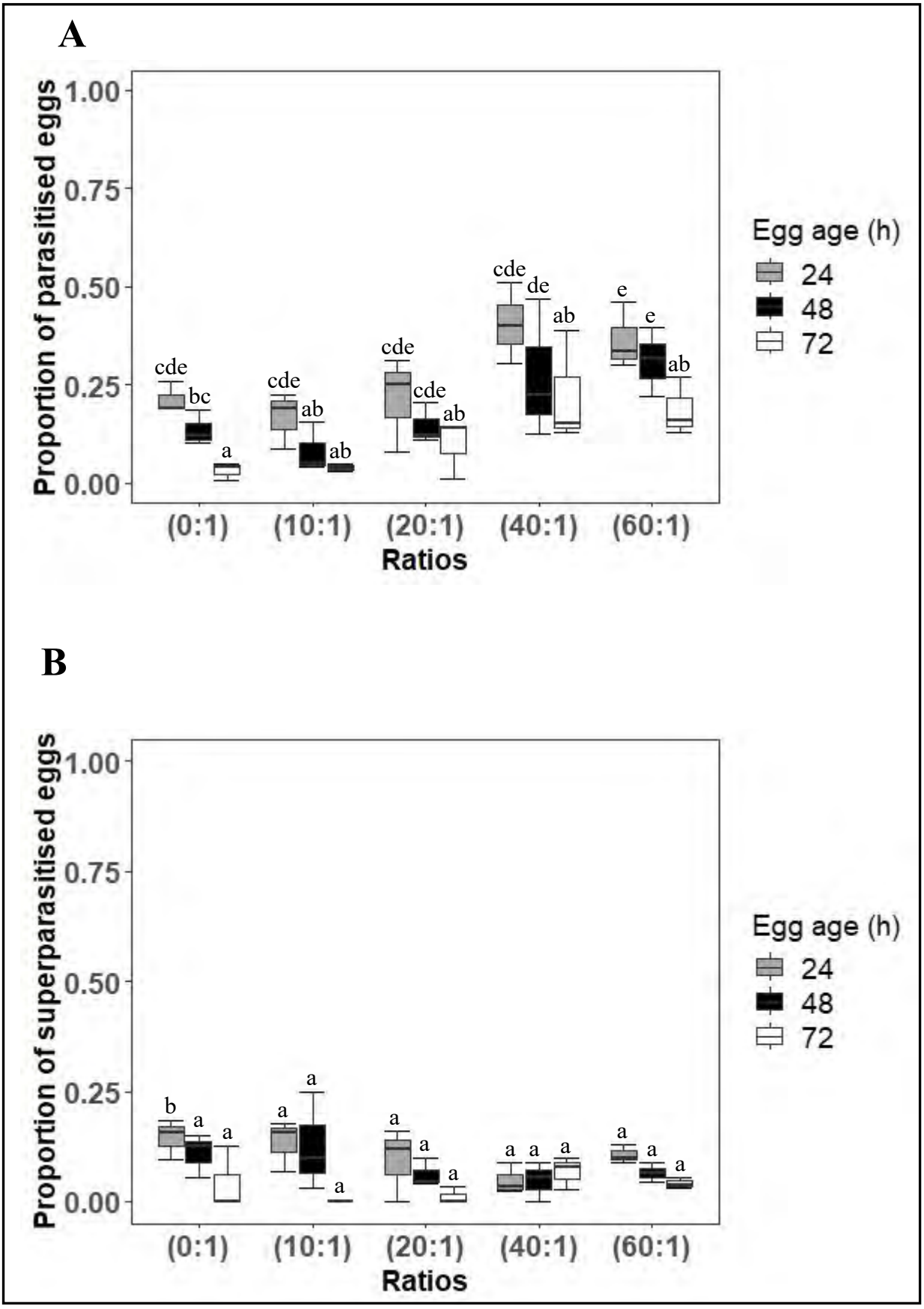
A
Proportion of parasitised eggs
1.00
0.75
0.50
0.25
0.00
cde
bc
a
cde
ab
ab
cde
cde
ab
cde
de
ab
e
e
ab
(0:1)
(10:1)
(20:1)
(40:1)
(60:1)
Ratios
Egg age (h)
24
48
72
B
Proportion of superparasitised eggs
1.00
0.75
0.50
0.25
0.00
b
a
a
a
a
a
a
a
a
a
a
a
a
a
a
(0:1)
(10:1)
(20:1)
(40:1)
(60:1)
Ratios
Egg age (h)
24
48
72

Fig. 5.1: The proportion of parasitised **A**, and superparasitised eggs **B** as affected by different ratios (sterile to fertile *T. leucotreta*) and age of the *T. leucotreta* eggs. Boxplots show median values (solid lines), and whiskers show the range of the data. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

The proportion of emerged egg parasitoids was significantly influenced by the different ratios ($\chi^2 = 45.75$; $df = 4$; $P < 0.05$). Similarly, egg age also had a significant effect on the proportion of emerged egg parasitoids across all the different ratios ($\chi^2 = 57.33$; $df = 2$; $P < 0.05$). Notably, there was a significant difference in the proportion of emerged egg parasitoids between 0:1 and 40:1 when exposed to 72 h old eggs (Fig. 5.2A). In addition, at the egg ages of 24 and 48 h, the proportion of emerged egg parasitoids was significantly different from those at the egg age of 72 h, regardless of the ratios. The interaction between the different ratios and egg ages did not have a statistically significant impact on the proportion of emerging egg parasitoids ($\chi^2 = 14.69$; $df = 8$; $P = 0.07$).

The emerging parasitoid female-to-male sex ratio displayed some variation across different ratios and *T. leucotreta* egg ages (Fig. 5.2B). In the ratio of 0:1, parasitoid females-to-male sex ratio decreased as the egg age increased. Similarly, this was the trend at the ratio of 10:1. Conversely, at the ratio of 60:1, parasitoid females-to-males sex ratio marginally increased with an increase in egg age. Fluctuations in the sex ratio were recorded across egg ages at ratios 20:1 and 40:1, such that more females than males emerged at egg ages 24 and 48 h, respectively. Subsequently, at the ratio of 60:1, more female to male parasitoids emerged at the egg age of 72 h. This differed from the ratio of 40:1, where more males to females emerged at the same egg age.

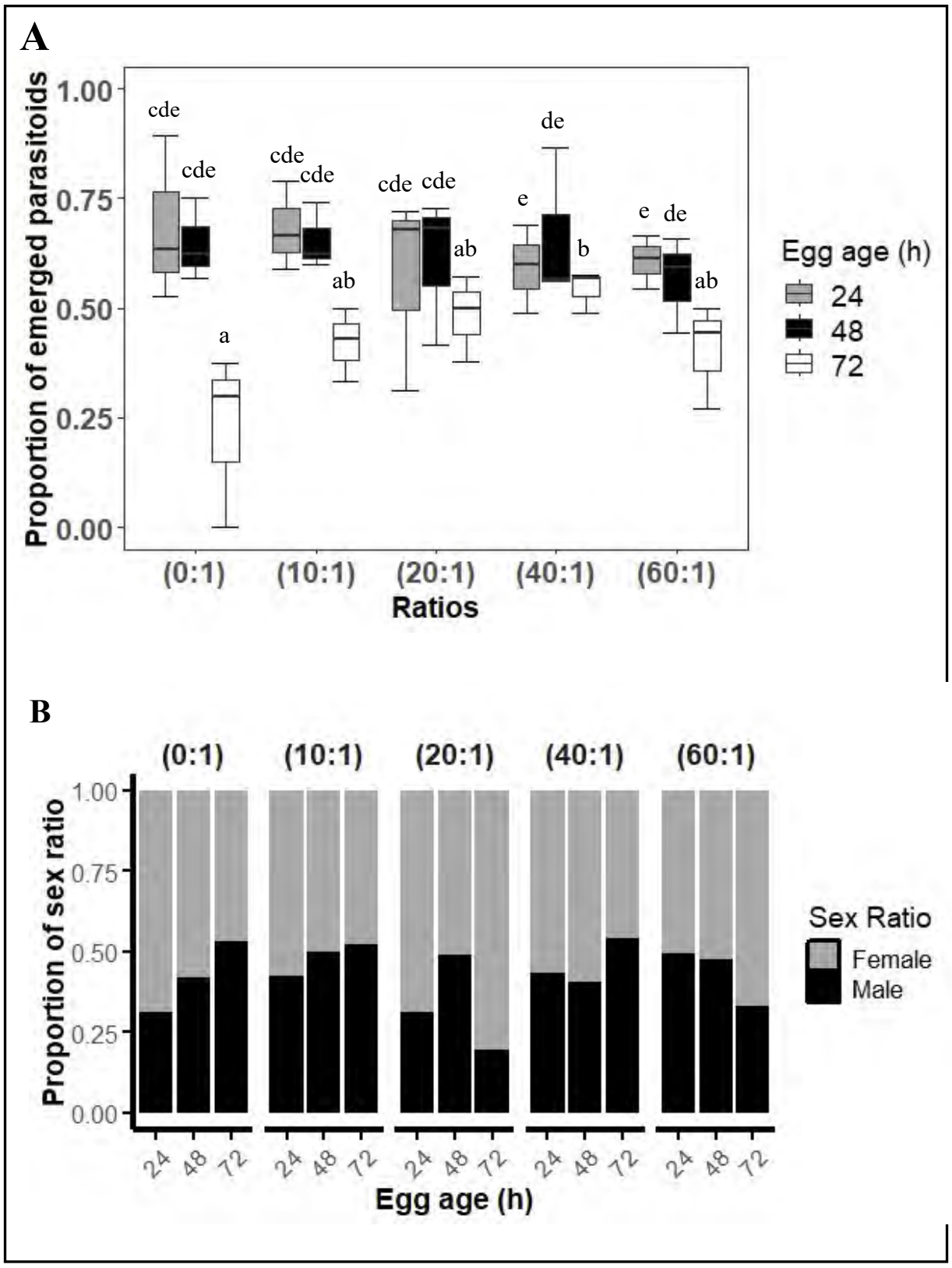

Fig. 5.2: The proportion of egg parasitoid emergence **A**, and the sex ratio of emerged egg parasitoids **B** as affected by different ratios (sterile to fertile *T. leucotreta*) and age of the *T.*

leucotreta eggs. Boxplots show median values (solid lines), and whiskers show the range of the data. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

5.3.2 Parasitoid quality

The proportion of flying egg parasitoids that emerged from *T. leucotreta* eggs was significantly affected by the varying ratios (χ^2 =20.94; df = 4; $P < 0.05$). Additionally, the different ages of *T. leucotreta* eggs also had a significant influence on the proportion of flying egg parasitoids (χ^2 =146.13; df = 2; $P < 0.05$). Notably, there was a significant difference in the proportion of flying egg parasitoids between ratios 10:1 and 40:1, as well as between 20:1 and 40:1 at the egg age of 48 h. Additionally, at the egg ages of 24 and 48 h, the proportion of flying egg parasitoids was significantly different from the proportion of flying egg parasitoids at the egg age of 72 h across all ratios (Fig. 5.3A). Moreover, the proportion of flying egg parasitoids was significantly different across the three egg ages at the ratio of 20:1. The interaction between different ratios and *T. leucotreta* egg ages did not yield any significant differences (χ^2 = 6.62; df = 8; P = 0.58).

The proportion of emerged flying female-to-male parasitoid sex ratio displayed some variation concerning different ratios and *T. leucotreta* egg ages (Fig. 5.3B). At a ratio of 0:1, a high proportion of females flying egg parasitoids were recorded. At the ratios of 10:1, 40:1, and 60:1, female-to-male sex ratio marginally decreased with an increase in egg age. However, at the ratio of 20:1, a high proportion of flying males was recorded at the egg age of 72 h.

The proportion of walking egg parasitoids that emerged was significantly affected by the different ratios (χ^2 = 74.62; df = 4; $P < 0.05$). (Fig. 5.4). Additionally, the different egg ages significantly affected the proportion of walking egg parasitoids emerging from the *T. leucotreta* eggs (χ^2 = 17.44; df = 2; $P < 0.05$). There was a significant increase in the proportion of walking egg parasitoids between the ratio of 0:1 and 40:1 as well as 0:1 and 60:1 at egg ages of 24 and 72 h (Fig. 5.4). The interaction between different ratios and *T. leucotreta* egg ages did not result in any significant differences (χ^2 = 13.20; df = 8; P = 0.11).

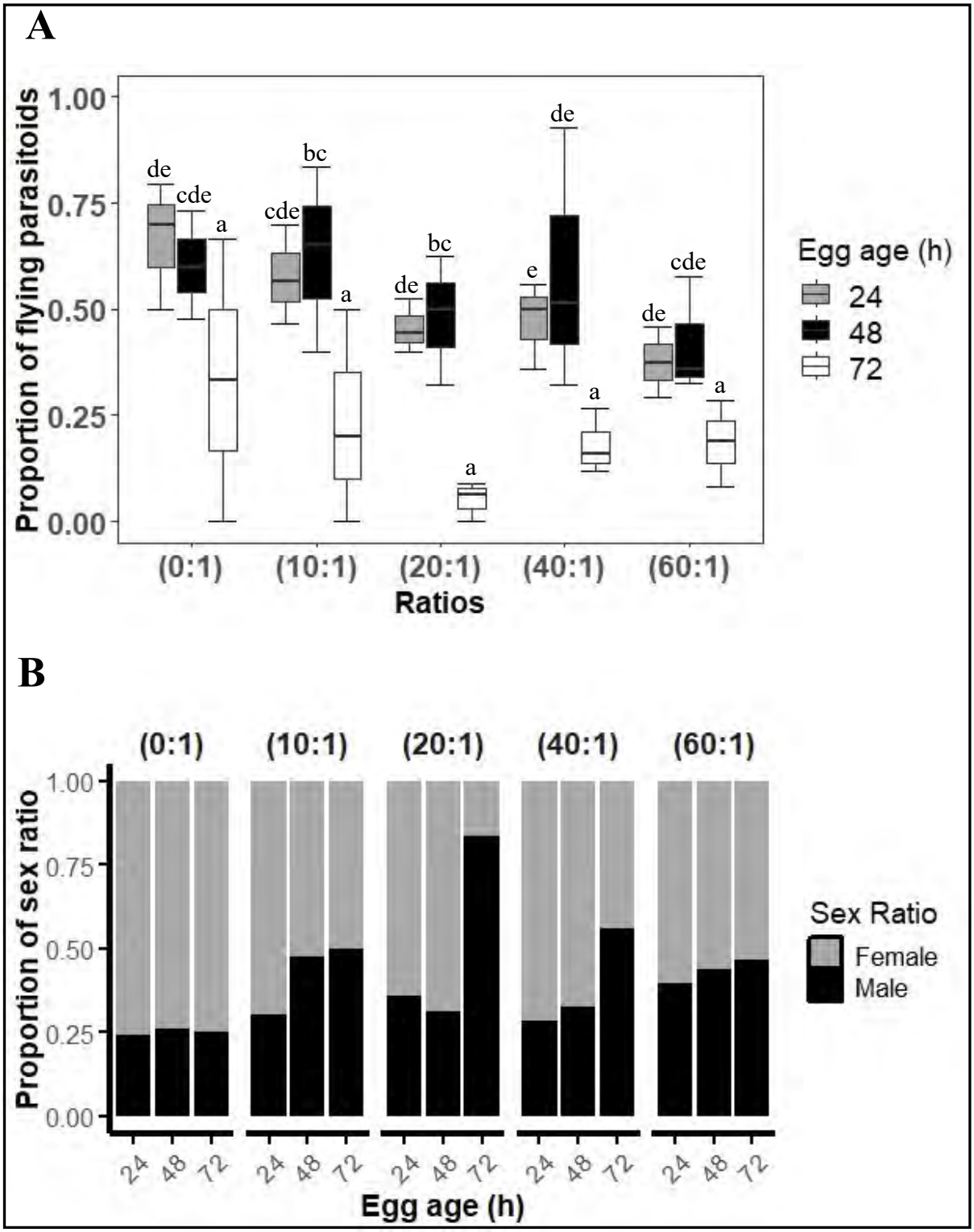

Fig. 5.3: The proportion of flying egg parasitoids **A**, and the sex ratio of emerged flying egg parasitoids **B** as affected by different ratios (sterile to fertile *T. leucotreta*) and age of the *T. leucotreta* eggs. Boxplots show median values (solid lines), and whiskers show the range of the data. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

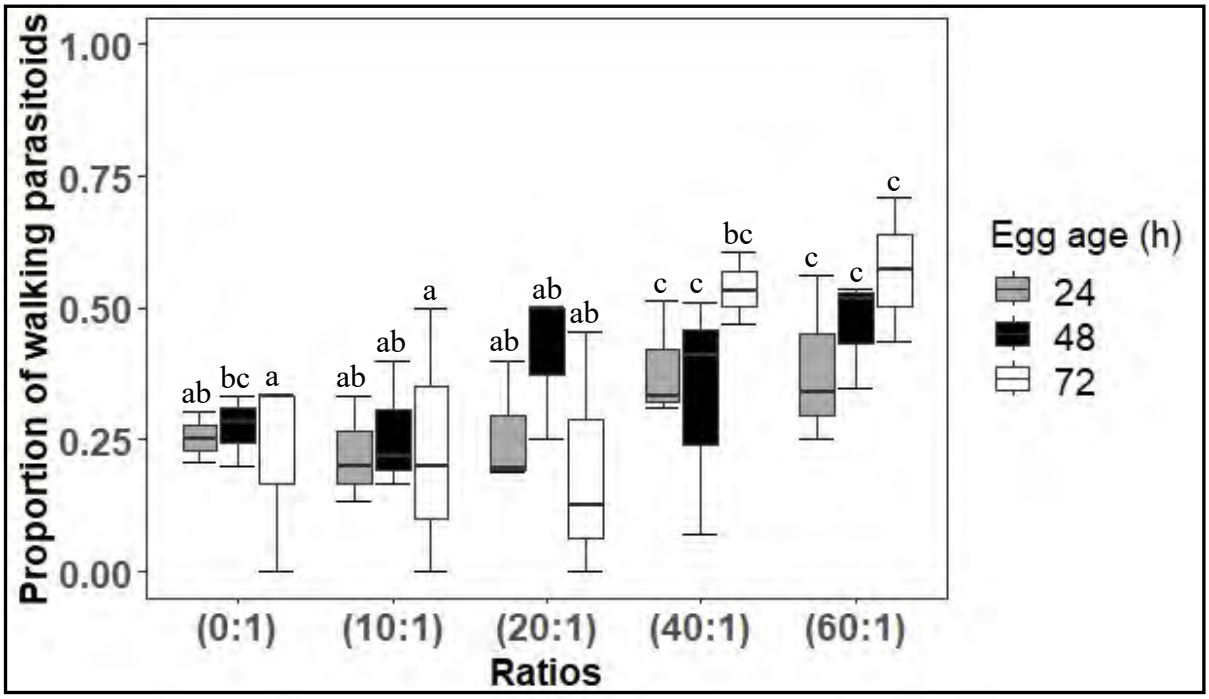

Fig. 5.4: The proportion of emerged walking egg parasitoids as affected by different ratios (sterile to fertile *T. leucotreta*) and age of the *T. leucotreta* eggs. Boxplots show median values (solid lines), and whiskers show the range of the data. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

5.4 Discussion

In a biological control programme incorporating egg parasitoids, the acceptance, and suitability of host eggs by the parasitoids for their development is crucial for effective pest control (Carpenter et al., 2004; Kaspi et al., 2020). In this study, the parasitism levels of the egg parasitoid, *T. cryptophlebiae* against *T. leucotreta* eggs produced from different sterile to fertile ratios were determined. The results indicated that all *T. leucotreta* eggs laid from different ratios and exposed to female egg parasitoids were acceptable for oviposition, demonstrating their ability to support egg parasitoid development. This finding aligns with a study by Carpenter et al. (2004), which showed that egg parasitoids can successfully develop and emerge from eggs originating from different crosses in the field.

From this study, it is evident that both the different ratios of sterile to fertile moths and the egg age did influence the parasitism rate, with higher levels of parasitism occurring in the younger eggs compared to the older eggs. A significant proportion of *T. leucotreta* eggs were parasitised at the egg age of 24 and 48 h compared to 72 h, across the ratios, except for 10:1. Host fertility, host age, and the contact time between host and parasitoid, play a key role in parasitism (Cossentine et al., 1996; Makee, 2006). Therefore, the decrease in parasitism rate with increasing egg age in the current study may be attributed to the declining suitability of host eggs as they age, given that more nutrients are used up by the developing *T. leucotreta* larva (El Sharkawy, 2011; Peñaflor et al., 2012), which aligns with the findings of Carpenter et al. (2004), indicating a similar trend of decreasing parasitism rate with increasing *T. leucotreta* egg age.

The results suggest that a combination of different ratios of sterile to fertile *T. leucotreta* and egg parasitoids could provide an additional suppressive effect against *T. leucotreta*, given the acceptability and suitability of the eggs for oviposition by the egg parasitoids. This observation corresponds with studies by Nagy (1973), Cossentine et al. (1996), and Botto & Glaz (2010), which found that combining the release of sterile insects with *Trichogramma* spp. egg parasitoids achieved a suppressive effect on *C. pomonella*. Similarly, Bloem et al. (1998) suggested that combining both control techniques yielded better results than using either method alone. The lack of feasible sexing methods in most SIT programmes for lepidopteran pests, results in various mating events, including those involving irradiated moths, which produce non-viable eggs (Marec & Vreysen, 2019). These non-viable eggs can support parasitoid build-up, by providing additional host material in the field (Nagy, 1973; Cossentine et al., 1996; Bloem et al., 2007; Marec & Vreysen, 2019). Moreover, *T. leucotreta* eggs that were not parasitised by the egg parasitoids may either fail to hatch if they originate from a sterile *T. leucotreta* female or may develop into sterile F_1 adults if they originate from a sterile male-fertile female pairing, contributing to further suppression of *T. leucotreta* (Bloem et al., 2003). This is because the radiation dose used completely sterilises the female *T. leucotreta*, while the males are partially sterile, resulting in the production of progeny that is both completely sterile (F1 or inherited sterility) and strongly biased towards males (Bloem et al., 2003).

Superparasitism was not greatly affected by the different ratios and the age of *T. leucotreta* eggs. A higher proportion of superparasitism was recorded in the ratio of 0:1 at an egg age of 24 h, but this proportion decreased significantly at the egg age of 48 and 72 h. This decrease in superparasitism can be attributed to the lower quality of host eggs, resulting

from crosses between sterile males and females, which may be unsuitable for the development of two or more parasitoids compared to high-quality eggs from fertile moths (Carpenter et al., 2004; Moreira et al., 2009).

The different ratios and egg age did influence the oviposition and emergence of egg parasitoids, with the younger eggs being preferred and, therefore, more suitable for egg parasitoid development compared to the older eggs. This finding aligns with studies by Reznik & Umarova (1985; 1990), El Sharkawy (2011), Peñaflor et al. (2012), and Bari et al. (2016), which suggested that the proportion of emerging egg parasitoids decreases as the age of the host egg increases, due to competition for resources with the developing host embryo. The chemical composition of the host egg changes as nutrients are gradually taken up by the host embryo, and the variations in the physical characteristics of the chorion, which hardens as the egg ages (Peñaflor et al., 2012). Similar results were reported by Pizzol et al. (2012), showing higher emergence rates of *Trichogrammatoidea cacoeciae* (Marchal) (Hymenoptera: Trichogrammatidae) obtained in 1-day-old host eggs compared to older hosts. However, these findings differ from Carpenter et al. (2004), who reported a significant reduction in the parasitoid emergence from 24 h old host eggs laid by sterile *T. leucotreta* compared to that from older eggs. This could be a result of the host egg being too young, resulting in low nutrient availability to support parasitoid development or leading to superparasitism, which ultimately results in parasitoid mortality (Reudler et al., 2007; Pizzol et al., 2012; Zhu et al., 2014). Nevertheless, the significant difference disappeared when the egg age was 48 h and 72 h. This could be attributed to the low preference of female parasitoids for host eggs laid from matings between sterile moths, resulting in a low level of parasitism and, hence, poor emergence of the parasitoids (Zhu et al., 2014).

There was variation in the female-to-male sex ratio of the emerging egg parasitoids, across different ratios and *T. leucotreta* egg ages. The different ratios did not affect the sex ratio of the egg parasitoids, as the egg age did, especially the young egg ages, where more females than males emerged. These results indicate that the majority of host eggs from sterile moths and older than 72 h are least preferred for oviposition, leading to the observed sex bias. This is crucial since female egg parasitoids are responsible for parasitism and population build-up in the citrus orchards. This finding conforms with the findings of Godin and Boivin (2000), which show host acceptance, successful parasitoid development, and that the fecundity of emerging females declines with increasing host age. According to Schmidt (1994), the clutch size and the progeny sex ratio are influenced by the female parasitoids' assessment of host age, size, and nutrient availability. This concept is in line with Charnov et

al. (1981) host quality model, which predicts that female parasitoids should produce a high proportion of males in low-quality host eggs, such as small and old host eggs, and a high proportion of females in high-quality host eggs. The males are allocated to the least preferred hosts (El Sharkawy, 2011).

According to Prezotti et al. (2002), to maintain the high-quality standards required for egg parasitoids, longevity, flight activity, and parasitism index need to be assessed. This approach is simpler than the evaluation of seven different biological variables in the laboratory, as recommended by the International Organization of Biological Control (IOBC Global Working Group: Quality Control of Mass-Reared Arthropods) (van Lenteren, 2003). In our flight tests conducted to assess the quality of the egg parasitoids emerging from *T. leucotreta* eggs produced from different ratios of sterile and fertile *T. leucotreta*, egg parasitoids were categorised into two groups: flyers and walkers, depending on their location after release in a flight test chamber, and egg parasitoids quality was assessed. The different ratios and egg ages significantly affected the proportion of flying and walking egg parasitoids after emergence. The majority of flying egg parasitoids emerged from young-age host eggs (24 and 48 h) compared to older host eggs (72 h). With the high emergence rate of flying egg parasitoids from *T. leucotreta* host eggs, the egg parasitoids can be effectively used as biological control agents in combination with SIT. Conversely, there was a significantly higher proportion of walking egg parasitoids emerging at the egg age of 72 h, in the ratios of 40:1 and 60:1 compared to 20:1, 10:1, and 0:1. Therefore, flight and walking are crucial locomotory aspects of trichogrammatid wasps, which aid in their dispersion in the field during foraging for food, hosts, and mates (Dutton & Bigler, 1995; Soares et al., 2012).

Consequently, the emergence of more flying egg parasitoids will be beneficial, as they are more mobile and can search and parasitise more *T. leucotreta* eggs, even in areas not easily accessible by other control techniques, such as chemical sprays (Pérez-Staples et al., 2021). There was a variation in the proportion of emerging flying egg parasitoid sex ratio, across different ratios and *T. leucotreta* egg ages. At the ratio of 0:1, the proportion of flying female egg parasitoids was higher than that of male egg parasitoids in all egg ages. The trend was also evident in other ratios, with more egg parasitoids emerging at egg ages of 24 and 48 h compared to 72 h. This female-biased sex ratio is beneficial in a biological control programme, as a few males can fertilize many females, contributing to parasitoid population development (King, 1987). Species with high emergence rates, high proportion of females, and high flight capacity can disperse faster in the field, locate hosts, and produce a high

number of progeny in shorter periods, aiding in more effective pest suppression (Soares et al., 2012).

Currently, the benchmark ratio of sterile to fertile *T. leucotreta* used in the SIT programmes on citrus orchards is 10 sterile males to 1 fertile male (Hofmeyr et al., 2005; Hofmeyr et al., 2015). This ratio was shown to be effective in research trials conducted to determine what overflooding ratio to use (Hofmeyr et al., 2005; Hofmeyr et al., 2015). However, the findings from Chapter 2 indicate that ratios from 40:1 can be more efficacious than a 10:1 ratio, with the 60:1 ratio resulting in the lowest fruit damage, larval entries, and emerging F1 adults. Bloem et al. (2003) and Hofmeyr et al. (2005) results convinced the South African citrus industry to adopt the technique and expedite the commercial production of the sterile moths through the establishment of XSIT. However, to mitigate the negative impacts of *T. leucotreta* on the citrus industry in South Africa, it is essential to implement additional management strategies that are efficient, effective, and environmentally friendly. Therefore, the combination of high ratios of sterile to fertile *T. leucotreta* and the biological control agent, *T. cryptophlebiae*, can provide some additive suppressive effects. These products can also potentially be supplied at a reduced cost where simultaneous production occurs, as done by River Bioscience. This company uses the excess *T. leucotreta* from XSIT for mass rearing of egg parasitoids, productively using the *T. leucotreta* eggs that would otherwise be discarded. This innovative approach contributes to cost-effectiveness and sustainability in the control of *T. leucotreta*. For full integration of SIT and augmentative releases of egg parasitoids to be effective in pest management, it is crucial to ensure that egg parasitoids do not undermine the efficacy of SIT programme (Carpenter, 1993; Carpenter et al., 2004).

This study indeed showed that the released sterile insects do not negatively affect the efficacy of the egg parasitoids. Therefore, the eggs produced from matings involving released sterile *T. leucotreta* can provide additional host material for egg parasitoid population build-up. Our finding suggests that high *T. leucotreta* ratios, greater than the current benchmark (10:1), can support the successful development of egg parasitoid populations and can be effectively combined for *T. leucotreta* control. However, further research is warranted to thoroughly examine the effectiveness of integrating these two strategies for *T. leucotreta* control under field conditions. Such studies will provide valuable insights and pave the way for more robust and efficient pest management practices in South Africa's citrus industry.

CHAPTER 6

Assessment of pre-release mating levels of *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) from moth emergence to the release stage in the sterile insect technique programme

[*]**Formatted as per Insect Science as** "Githae, M. M., Coombes, C. A., Mutamiswa, R., Moore, S. D., & Hill, M. P. (2024). Assessment of pre-release mating levels of *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) from moth emergence to the release stage in the sterile insect technique programme"

6.1 Introduction

Thaumatotibia leucotreta is endemic to SSA, the Ethiopian region, and several African islands, making it a key phytosanitary pest in export markets (Huisamen et al., 2022; EFSA, 2024). *Thaumatotibia leucotreta* is an important polyphagous pest, attacking over 70 host plants in 40 families (see Adom et al., 2021, Azrag et al., 2021). Female *T. leucotreta* lay eggs on the fruit rind, and the neonates, after hatching, burrow into the fruit, feeding on the pulp (Moore, 2022). This feeding behaviour causes premature fruit abscission, resulting in reduced yields, and can inflict notable losses in fruit export markets due to consignment rejections in the event that any live specimens are intercepted (USDA, 2010; SA-DAFF, 2015; EU, 2017; Mkiga et al., 2019). As a result, management of the pest populations below economic injury levels is imperative in citrus groves (Moore & Hattingh, 2012; Moore, 2022).

The use of conventional insecticides is a widespread practice for the control of *T. leucotreta* in SSA (Nepgen et al., 2018). However, this strategy is unsustainable, due to the development of insecticide resistance, the decimation of natural enemies and pollinators, and its hazardous effects on human and environmental health (Simmons et al., 2010; Azrag et al., 2021). In South Africa, an integrated control approach against *T. leucotreta* has been developed, which includes orchard sanitation, attract-and-kill techniques, mating disruption, biological control, and sterile insect techniques (see Foster & Harris, 1997; Moore & Hattingh, 2012; Hofmeyr et al., 2015; Boersma, 2021; Huisamen et al., 2022; Moore, 2022; Githae et al., 2024). As a result of stringent regulations imposed by export markets, regarding maximum residual limits (MRLs) of pesticides on exported fruit and concerns about the phytosanitary status of *T. leucotreta*, the commercialisation of the SIT in South Africa was facilitated in 2007 (Simmons et al., 2010; Hofmeyr et al., 2015; 2016). This was prompted by promising results from a series of trials that convinced the citrus industry in the country of the efficacy and viability of the approach (see Bloem et al., 2003; Carpenter et al., 2004; Hofmeyr et al., 2005; 2015).

SIT and its derivative, F1 sterility, constitute a vital element in the AW-IPM strategy for countering *T. leucotreta*, as it is species-specific and environmentally benign (Simmons et al., 2010; Hofmeyr et al., 2015). This holistic approach encompasses the large-scale generation of sterile moths (50 million *T. leucotreta* per week), which are produced in the XSIT (Pty) Ltd. mass-rearing facility situated in Citrusdal, South Africa, and are then distributed to citrus orchards, spanning about 19500 hectares across the Western Cape, Eastern Cape, and Northern Cape Provinces of South Africa (XSIT, 2018; Boersma, 2021;

Huisamen et al., 2022). In citrus orchards, the sterile *T. leucotreta* are released using helicopters (Hofmeyr et al., 2015).

Several variables can influence the strategies adopted in SIT programme for the management and deployment of sterile insects (Dowell et al., 2005), and these choices can significantly influence the quality and effectiveness of the released sterile insects (Calkins & Parker, 2005). Ensuring the supply of high-quality sterile moths mandates meticulous oversight of production, handling, processing, transportation, and release protocols. This helps to ensure that the mating competitiveness of the sterile *T. leucotreta* against fertile wild populations can be maintained (Boersma, 2021). Various factors, such as climate, food availability, and mating behaviour, i.e., frequency and duration, affect the reproduction of insects (Gullan & Cranston, 2014). Nevertheless, due to the impracticability of segregating sterile male and female *T. leucotreta*, dual-sex releases were adopted (Hofmeyr et al., 2015). Furthermore, the release of both sterile males and females has been shown to improve the efficacy of the SIT system, relative to males-only releases (van Steenderen, 2017). The findings in Chapter 3 indicated that dual-sex releases can result in some *T. leucotreta* suppression if conducted over several generations compared to the untreated *T. leucotreta*. However, in other pests such as Medflies, only sterile male releases are recommended, due to the damage that can still be inflicted by sterile females to the crops (Calkins & Parker, 2005).

Nevertheless, with female *T. leucotreta* being polyandrous (Azrag et al., 2021) and males having the ability to be polygynous (Trivers, 2017; Azrag et al., 2021), to maximise their reproductive success, control of sterile male and sterile female matings (negative sperm sink) is crucial. This reduces the number of mating incidents of sterile males with wild females, or sterile females might mate more frequently with wild males (positive sperm sink) (Calkins & Parker, 2005). However, multiple matings require more energy and time, lowering the insect's mating vigour, imposing risks to the females due to the physical injuries during male manipulation and mating, and the transmission of pathogens (Cingolani et al., 2020). Moreover, multiple matings can have a negative effect on egg production, fertility, and lifespan in some species (Arnqvist & Nilsson, 2000). However, to minimise this in *T. leucotreta*, moths are maintained at a temperature of 6 to 8°C (Nepgen et al., 2015; Boersma, 2021). This also aids in easier handling, packaging, and transporting of the sterile insects (Huisamen et al., 2022).

However, unintended pre-release matings are associated with risks that can potentially undermine the effectiveness of the technique. Hence, it becomes imperative to scrutinise the levels of pre-release mating between sterile male and female *T. leucotreta* before release,

tracing the mating through various stages of production and handling up to the release stage. Therefore, the study investigated pre-release mating that occurs across the different stages of sterile *T. leucotreta* production and release. The results obtained are discussed in the context of improving *T. leucotreta* pest suppression by the production of high-quality sterile *T. leucotreta* males that are competitive with the fertile wild population when released in the citrus orchards, thus further improving the effectiveness of the SIT programme.

6.2 Materials and Methods

6.2.1 Insect rearing, handling and processing

The production of *T. leucotreta* was divided into the following phases: egg production, diet preparation and inoculation, larval rearing and pupal harvesting, moth emergence and collection, and moth irradiation (Boersma & Carpenter, 2016; Boersma, 2021). The key components for this study were moth eclosion and collection, moth irradiation, and moth transportation and release.

6.2.1.1 Moth eclosion and collection

Moth eclosion was conducted in two rooms, with a combined floor area of 326 m^2. Inside these rooms, a total of 146 emergence cabinets were arranged in 13 rows (Fig. 6.1). Each cabinet measures 673 mm × 860 mm × 1678 mm and accommodates 50 pupation boards. These cabinets are divided into sections by a metal panel, which has small openings to permit newly emerged moths to pass from the dark compartment to the illuminated one. The temperature was maintained precisely at 25 ± 1°C. To collect the moths, there are 13 collection troughs, each aligned with a row of cabinets. To induce dormancy, the moths were maintained at a temperature of 9 ± 1°C. Once the moth count reached approximately 2 cm in the trough, they were emptied into baskets. The moths were then placed into cardboard boxes (130 mm × 130 mm), ready for transportation to the irradiation room.

Fig. 6.1: *Thaumatotibia leucotreta* emergence cabinets

6.2.1.2 Moth irradiation

The mass-rearing facility employs a 20 kCi ^{60}Co source panoramic irradiator to sterilise the moths. The source allows the placement of 8 canisters, with each canister able to hold 4 cardboard boxes containing 14,000 ± 200 moths. Moths were then irradiated with 180 Gy to induce sterility; however, this radiation dosage fluctuates from 150 to 200 Gy depending on the period of the season (Bloem et al., 2003). Packaging was conducted in a cold room maintained at 4°C to ensure that the insects remained inactive (Neville Boersma pers. comm.).

6.2.1.3 Moth transportation and release

After irradiation, the moths were placed in separate cages within a distribution room, each designated for deliveries to different regions. The ambient temperature in this room was maintained at 4 to 6 °C. The moths themselves were within a temperature range of 6 to 8°C, although their temperatures could be slightly higher inside the boxes. However, moths are not dispatched if their temperature is above 8°C (Neville Boersma pers. comm.). This is because higher temperature could lead to increased activity during transportation, potentially resulting in damaged and less effective moths, as well as unintended mating. Moths dispatched to the

Eastern Cape Province were loaded into a refrigerated truck as soon as truck's temperatures were within the required range. Throughout the transportation process, the moths' temperature was maintained at 6 to 8°C.

6.2.2 Experimental design

The trial was divided into different phases: moth eclosion and collection, moth irradiation, moth transportation, and release stage (Boersma & Carpenter, 2016). The trial was conducted in the warmer summer months of the year (January, March, and May).

Sample collection was conducted for the period moths were retained in the cabinets (~1 h), before being moved for sterilisation and after irradiation was conducted. *Thaumatotibia leucotreta* samples were randomly collected from 20 different cardboard boxes (30 mm × 70 mm × 110 mm) where a total of 20 female *T. leucotreta* were sampled per cardboard box at moth eclosion and post-irradiation stages. The collected samples were put in 90 ml round plastic containers (5 cm × 5 cm) (20 female moths per plastic container) and immediately moved into a deep freezer (845 mm × 644 mm × 1116 mm) to kill the moths and prevent any further degradation of the spermatophores if any mating had occurred. The samples were then transported by road to Rhodes University, Makhanda, South Africa (~ 840 km, 13-14 h) in a polystyrene cooler box containing dry ice bricks (4 to 6°C) for examination. In the laboratory, *T. leucotreta* samples were dissected using a stereomicroscope (Zeiss Microscopy, South Africa), at a magnification of 40× and the number of spermatophores in the bursa copulatrix was examined to determine if any mating had occurred (Lincango et al., 2013). The total number of mated *T. leucotreta* females per sample cardboard box was recorded.

After irradiation, the moths were transported in refrigerated trucks, with temperatures maintained at 6 to 8°C. On arrival in the Western Cape region at Olifants River Valley (ORV), about 2 h drive from the XSIT rearing facility, and in the Eastern Cape region at Sundays River Valley (SRV) about 10-12 h drive from the production facility, the moths were prepared for release aerially using a helicopter (Boersma, 2021). However, before this happened, *T. leucotreta* samples were randomly collected from different cardboard boxes where 20 female *T. leucotreta* were collected from 20 cardboard boxes from the insects delivered in both regions for field release. The collected *T. leucotreta* were placed in 90 ml round plastic containers, and frozen in a deep freezer, after which they were later transported to the laboratory for examination. In the laboratory, the *T. leucotreta* females were dissected using a stereomicroscope (as mentioned above), and the number of spermatophores in the

bursa copulatrix was examined to determine if any mating had occurred before the release. The total number of mated female *T. leucotreta* per sample cardboard box was also recorded.

6.2.3 Statistical analysis

Data collected from the study were checked for homogeneity of variance (F test, Levene's test) and normal distribution of residuals using the Shapiro Wilk test (Shapiro & Wilk, 1965). As parametric assumptions were not met, non-parametric tests were used. The data were analysed using a negative binomial generalised linear model analysis as an extension of the Poisson distribution to allow for count data with a significant proportion of zero values, with production stage and month as the sources of variation, as recommended by O'Hara and Kotze (2010). Analysis of deviance (log-likelihood ratio statistic) was used to assess the goodness of fit of the Poisson regression model, which has a distribution similar to that of chi-squared ($\chi 2$). The dependent variables used in the statistical model included the number of spermatophores recorded and the number of mated *T. leucotreta* females per production and release stage. Tukey-Kramer post-hoc comparisons were conducted at $P < 0.05$, where the statistical model indicated significant treatment effects and significant interactions. All statistical analyses were conducted using the R software version 4.3.1 (R Core Team, 2023).

6.3 Results

6.3.1 Overall pre-mating

The mean number of spermatophores recorded was significantly affected by the different production stages ($\chi 2 = 22.87$; df = 3; $P < 0.05$). Overall, the highest number of spermatophores were recorded at the irradiation stage (8.18 ± 1.78) followed by the release stage at the SRV region (4.83 ± 1.04), while the release stage at the ORV region (1.88 ± 0.44) recorded the lowest (Fig. 6.2). Significantly higher mean number of spermatophores were recorded at the irradiation stage compared to eclosion stage (3.52 ± 0.79) and release stage at ORV region (Fig. 6.2).

Likewise, the percentage of mated female *T. leucotreta* recorded was significantly affected by the different production stages ($\chi 2 = 18.13$; df = 3; $P < 0.05$). Overall, the highest percentage of the mated female *T. leucotreta* was recorded at the irradiation stage (16.83 ± 4.06% of mated females) while the lowest percentage was recorded at the ORV region release stage (3.75 ± 0.93% of mated females) (Fig. 6.3). Similarly, a significantly higher

difference was recorded at release stage at SRV region (9.21 ± 2.18% of mated females) compared to release stage at ORV region. Overall, no significant difference in the percentage of mated female *T. leucotreta* was recorded between the eclosion and irradiation stage as well as eclosion and both release stages (Fig. 6.3).

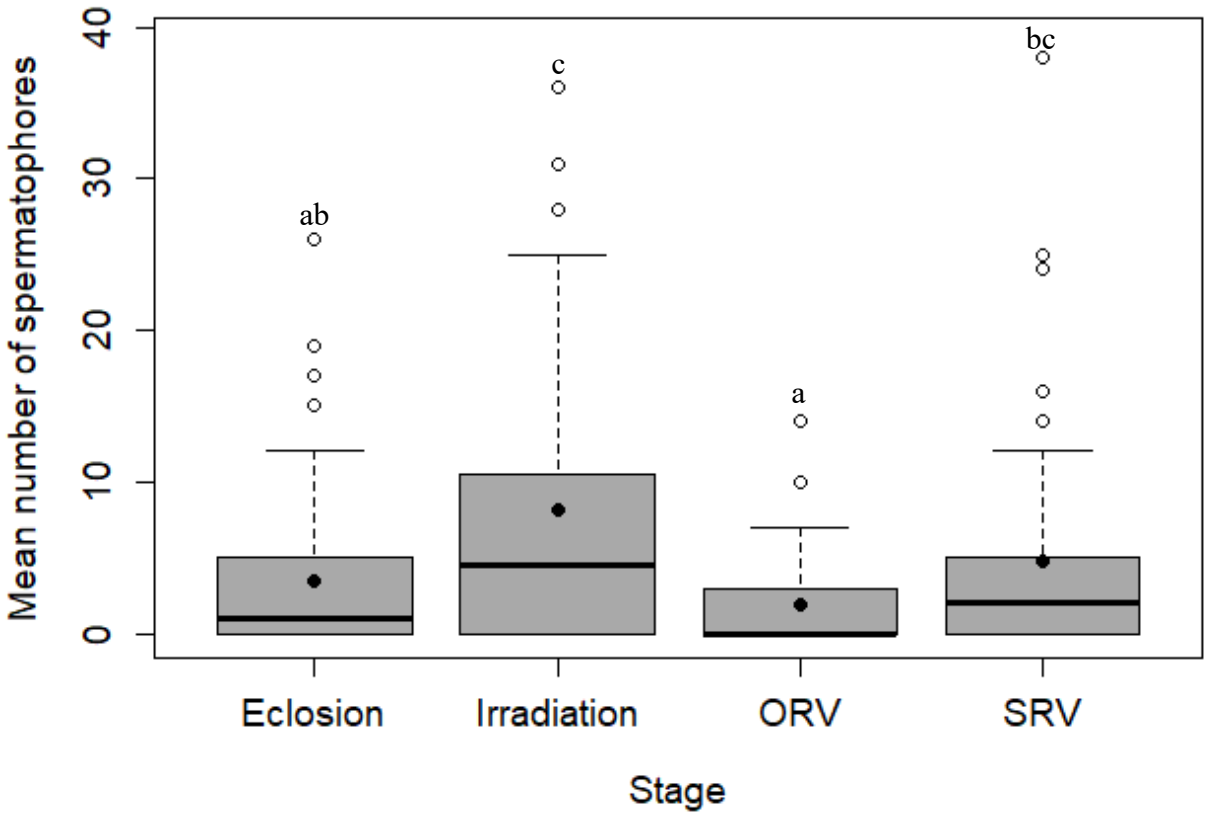

Fig. 6.2: Overall mean number of spermatophores per 20 female *T. leucotreta* from different production stages of sterile *T. leucotreta* and release regions. ORV and SRV represent Olifants River Valley and Sundays River Valley, respectively. Grey boxes show the interquartile range, and whiskers show the range of the data. The black dots indicate the mean number of spermatophores in the mated female *T. leucotreta* per production stage and release regions, while the white dots represent the outliers. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

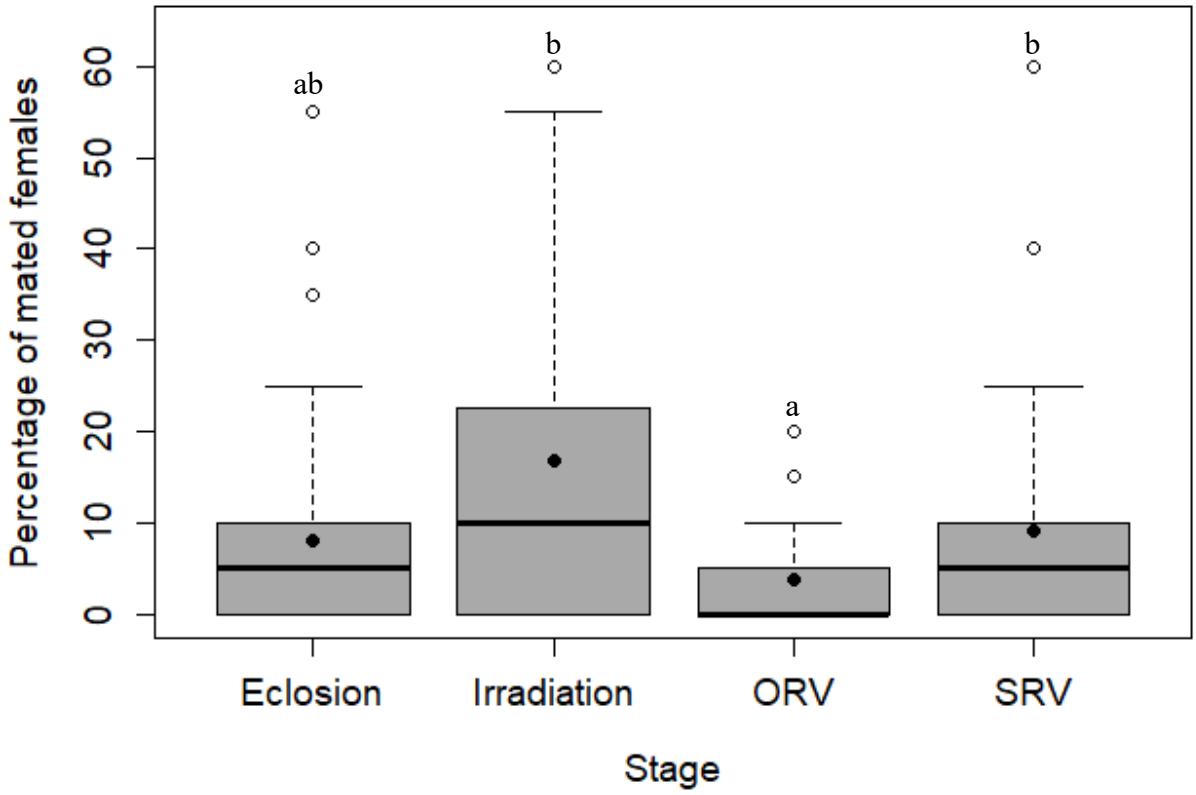

Fig. 6.3: Overall percentage of mated female *T. leucotreta* from different production stages of sterile *T. leucotreta* and release regions. ORV and SRV represent Olifants River Valley and Sundays River Valley, respectively. Grey boxes show the interquartile range, and whiskers show the range of the data. The black dots indicate the mean percentage of mated female *T. leucotreta* per production stage and release regions, while the white dots represent the outliers. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

6.3.2 Mating across months

The mean number of spermatophores per 20 females were significantly affected by the different production stages ($\chi2 = 21.97$; df = 3; $P < 0.05$) (Fig. 6.4). In addition, the interaction between the different production stages and time of the year (month) significantly affected the mean number of the spermatophores recorded ($\chi2 = 27.07$; df = 6; $P < 0.05$). However, the time of the year did not significantly affect the mean number of spermatophores ($\chi2 = 0.44$; df = 2; $P = 0.80$). The highest mean number of spermatophores was recorded at the irradiation stage (14.20 ± 3.32 per 20 females) in January, while the lowest number (0.55 ± 0.25 per 20 females) was recorded at release stage in the ORV region, the same month (Fig. 6.4). Similarly, significantly higher mean number of spermatophores

were recorded at the irradiation and eclosion stage (14.20 ± 4.89 and 4.90 ± 1.74 per 20 females) in January compared to March (3.20 ± 1.16 and 4.20 ± 1.49 per 20 females), and in May (8.00 ± 2.72 and 1.45 ± 0.56 per 20 females), respectively. (Fig. 6.4). Likewise, a significantly higher mean number of spermatophores were recorded at the release region SRV in January (4.76 ± 1.98 per 20 females) and in May (8.00 ± 2.72 per 20 females), compared to March (2.76 ± 1.30 per 20 females). However, no significant difference was noted at the release stage in the ORV region across the months (Fig. 6.4).

The percentage of mated *T. leucotreta* females recorded was significantly affected by the different production stages ($\chi 2 = 16.53$; df = 3; $P < 0.05$) (Fig. 6.5). Likewise, the interaction between the different production stages and the time of the year significantly affected the percentage of mated female *T. leucotreta* recorded ($\chi 2 = 17.19$; df = 6; $P < 0.05$). However, the time of the year did not significantly affect the percentage of mated female *T. leucotreta* ($\chi 2 = 0.75$, df = 2; $P = 0.68$). The highest percentage of mated *T. leucotreta* females was recorded at the irradiation stage (29.00 ± 11.47% of mated females) in January, while the lowest percentage (2.25 ± 0.95% of mated females) was recorded at release stage in the ORV region, the same month (Fig. 6.5). Similarly, significantly higher percentage of mated *T. leucotreta* females were recorded at the irradiation and eclosion stage (29.00 ± 11.49% and 12.50 ± 4.98% of mated females) in January compared to May (15.25 ± 6.06% and 3.00 ± 1.24% of mated females) and in March (6.25 ± 2.52% and 8.50 ± 3.41% of mated females), respectively (Fig. 6.5). Likewise, significantly higher percentage of mated *T. leucotreta* females were recorded at the release region SRV in May (14.52 ± 5.63% of mated females) compared to March (7.38 ± 2.89% of mated females), and in January (5.71 ± 2.26% of mated females). However, no significant difference was noted at the release stage in the ORV region across the months (Fig. 6.5).

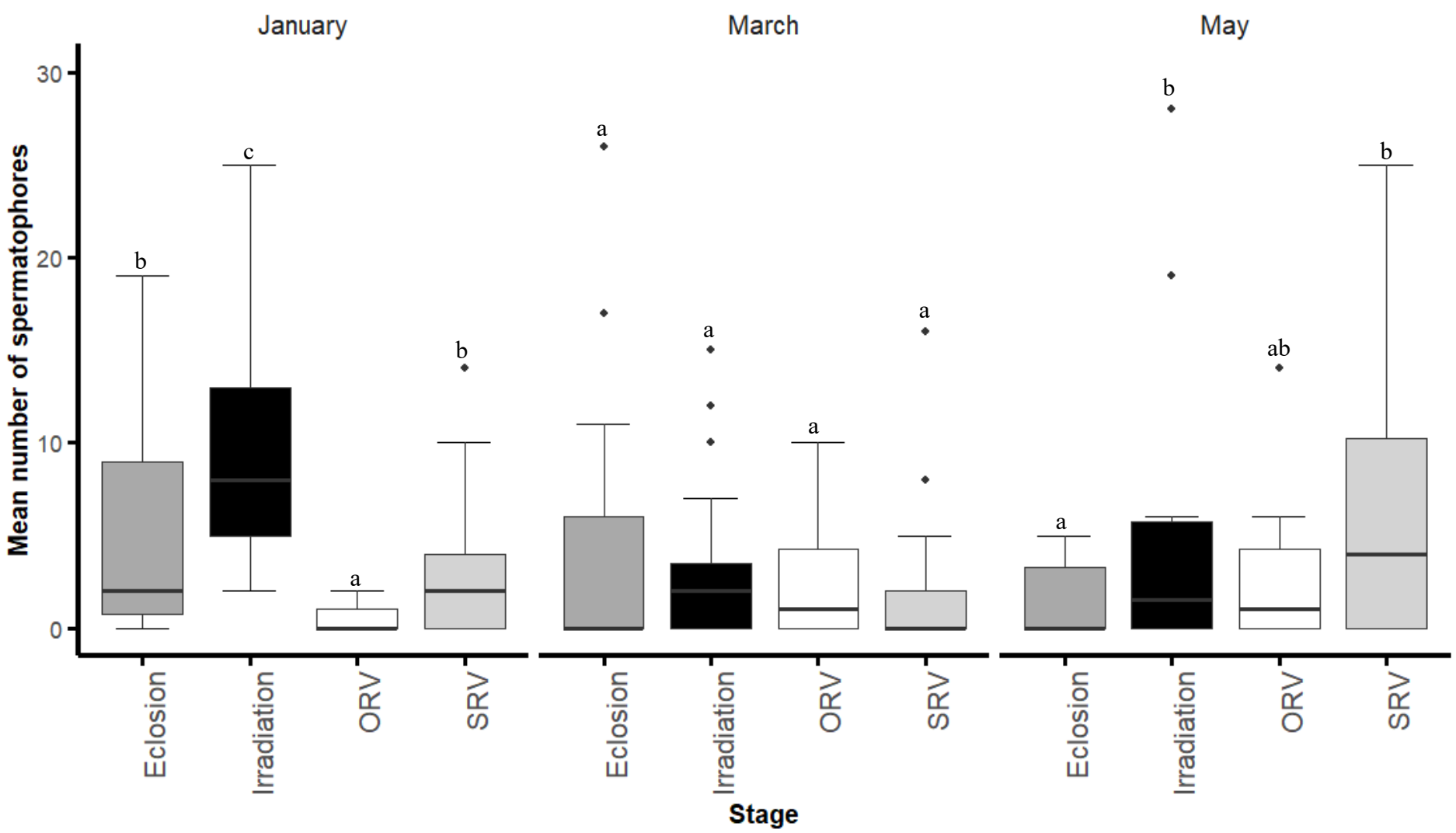
January
March
May
Mean number of spermatophores
30
20
10
0
b
c
a
b
a
a
a
a
a
b
ab
b
Eclosion
Irradiation
ORV
SRV
Stage

Fig. 6.4: The mean number of spermatophores per 20 female *T. leucotreta* from different production stages of sterile *T. leucotreta* and release regions in January, March, and May 2024. ORV represents Olifants River Valley, while SRV represents Sundays River Valley. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots represent the outliers. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

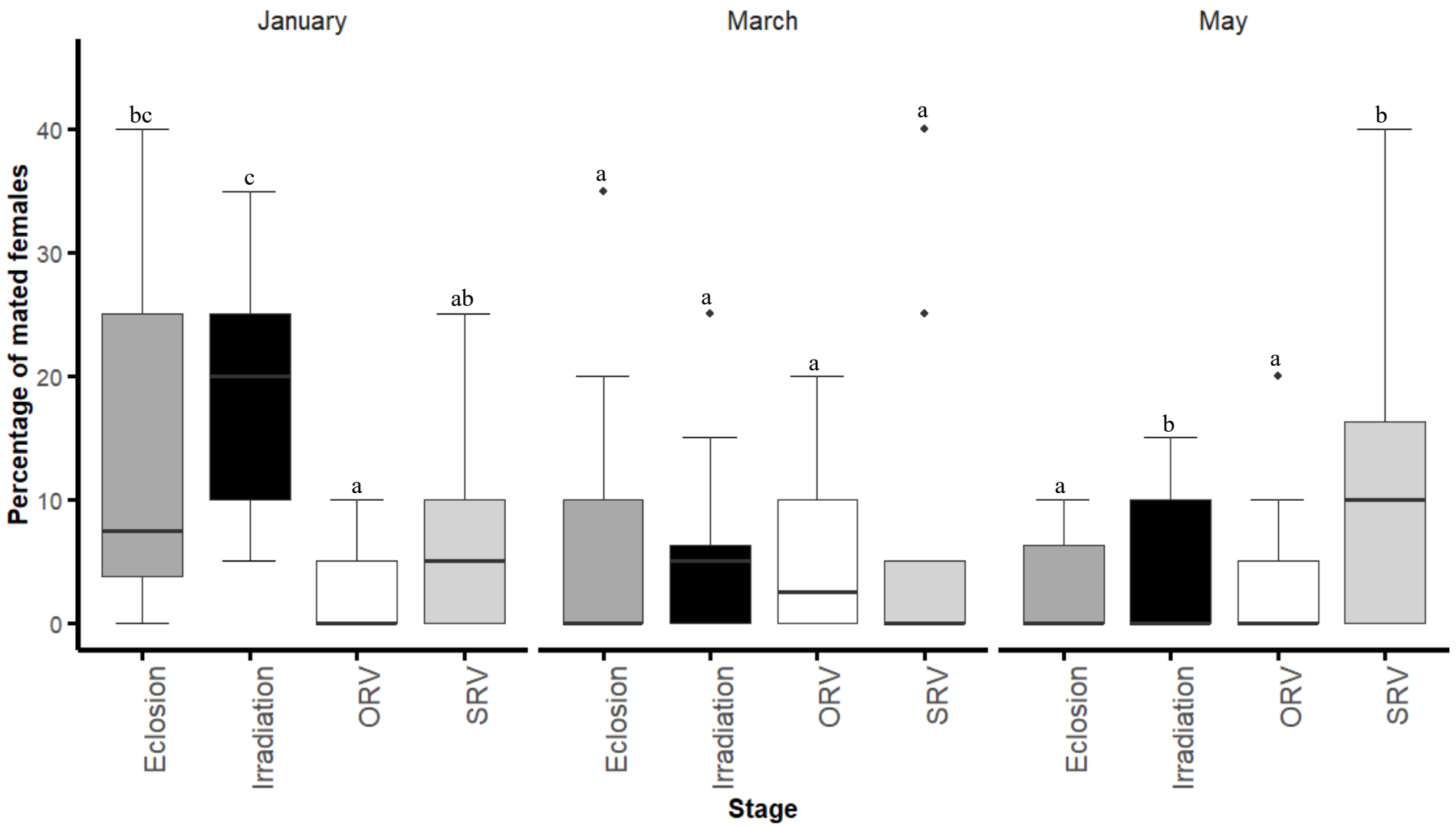
January
March
May
Percentage of mated females
0
10
20
30
40
bc
c
a
ab
a
a
a
a
a
b
a
b
Eclosion
Irradiation
ORV
SRV
Stage

Fig. 6.5: The percentage of mated female *T. leucotreta* from different production stages of sterile *T. leucotreta* and release regions in January, March, and May 2024. ORV represents Olifants River Valley, while SRV represents Sundays River Valley. Boxplots show median values (solid lines), and whiskers show the range of the data. The black dots represent the outliers. Different letters across the treatments indicate a statistically significant difference ($P < 0.05$).

6.4 Discussion

Sexual reproduction in insects is coordinated by multiple factors such as climatic conditions, availability of quality food, and mating behaviour of insects such as frequency and duration (Gullan & Cranston, 2014; Azrag et al., 2021). In most insect species, the frequency of the mating sorely influences their oviposition success, thereby affecting their population dynamics (Arnqvist & Nilsson, 2000). Mating is imperative for sexual reproduction; however, it can also pose risks to females, and in the majority of insects, females are polyandrous (Cingolani et al., 2020). Therefore, knowledge of monandry and polyandry matings is crucial in understanding how mating frequencies and reproductive success of species affect their population growth (Bakker et al., 2011).

From our overall findings, the irradiation stage and release stage in the SRV region had the highest mean number of spermatophores and the highest percentage of mated female *T. leucotreta*, while ORV had the lowest. The high mating incidences at SRV can be attributed to long-distance transportation, taking longer before moths are released which increases the chances of moths mating (Nepgen et al., 2015). The high mating incidences at the irradiation stage can be due to temperature fluctuations in the refrigerated room, maintained at 4°C, while the moth temperature was estimated to be ± 6°C to ensure that the insects remained inactive before loading into the delivery trucks (Boersma & Carpenter, 2016). The findings align with those of Chidawanyika and Terblanche (2011), demonstrating that the short-term fluctuations (diel thermal history), rate of temperature change, magnitude, and duration of exposure affected the survival of adult *C. pomonella*. Consequently, the fitness and performance of *C. pomonella* were affected, as the *C. pomonella* SIT programme used cold-induced immobilisation for the shipment of the moths. However, rigorous standard operating procedures for handling, packaging, and transporting the moths were later implemented (Chidawanyika & Terblanche, 2011).

Our study showed that the pre-mating occurs during both the production and release stages in different regions where SIT is implemented. A higher number of spermatophores and a greater percentage of mated female *T. leucotreta* were recorded at the irradiation stage followed by the eclosion stage in January and May. These findings align with those reported by Azrag et al. (2021) indicating that *T. leucotreta* becomes sexually active shortly after eclosion (6-13 h). Our findings indicate that pharate adults started mating after eclosion, and continued post-irradiation, through to the release stage. This confirms that sterile females can act as a negative sperm sink by mating with sterile males, potentially diminishing the technique's effectiveness (van Steenderen, 2017). This could reduce the mating events between sterile males and wild fertile females and between sterile females and wild fertile males (Calkins & Parker, 2005). Our study reported pre-mating at the moth eclosion stage. However, according to Boersma (2021), the adult moths in the collection pans should be kept immobile at 10°C to prevent any mating and avoid wing damage as mated adults or those with damaged wings may have a negative effect on their field performance. Despite no set or established temperature range during the moth collection, this could have resulted in the matings among the moths (Boersma, pers. comm.). The temperature at 10°C might have facilitated the mating incidences by making the moths active, resulting in spermatophore transfer among the sterile moths (Boersma, 2021). Barboza et al. (2023) demonstrated that remating could reduce the success of *C. capitata*, as the sperm of the second male may dominate that of the first. Consequently, the progeny of the female that remates with a wild male could be viable. Similarly, Ramírez-Santos et al. 2017 also demonstrated in mating and remating trials that *C. capitata* has a relative precedence in sperm use, where the sperm from remating male is used first.

According to Bakker et al. (2011), multiple matings in insects like *T. leucotreta* can negatively impact their fitness and with dual-sex releases conducted with sterile *T. leucotreta*, this can affect the success of the technique. This is due to the additional energy and time spent on mating, reducing the sterile *T. leucotreta* males and females' vigour in searching for and attracting wild fertile *T. leucotreta* for mating (Cingolani et al. 2020). Additionally, multiple matings endanger the sterile females due to physical injuries from male manipulation, and the transmission of infectious pathogens during mating (Gullan & Cranston, 2014; Cingolani et al., 2020). Similarly, matings between sterile males and sterile females can be prolonged, and long-lasting matings decrease the males' ability to find a wild fertile female *T. leucotreta* to mate with, thereby reducing the transfer of sterile spermatophores when released in citrus orchards (Vetter & Baker, 1990; Cingolani et al.,

2020). Nevertheless, de Villiers et al. (2019) demonstrated that females mated before irradiation were 10× more fertile than non-mated females, which could increase the chances of fruit infestation. However, these were preliminary results, and no meaningful conclusions were drawn.

In March, there was no significant difference in the mean number of spermatophores and the percentage of mated female *T. leucotreta* recorded across the different stages of production and release stage at ORV and SRV regions. This consistency can be attributed to the quality of the moths produced and the conditions at the facility and during transportation, which might have minimised mating incidences (Boersma, 2021). Typically, *T. leucotreta* SIT programmes use optimal rearing temperatures to maximise production regardless of post-release environmental conditions, but this approach may negatively impact the dispersal potential of the mass-reared insects upon release (Boersma et al., 2019). This is done through the use of cold temperatures to immobilise insects before release facilitating collection, handling, irradiation, transportation, and release of these insects (Dowell et al., 2005; Boersma et al., 2019). However, in May, a higher number of spermatophores and a greater percentage of mated female *T. leucotreta* were recorded at the release stage in the SRV region, and post-irradiation. The higher mating incidences before release at SRV can be attributed to the long distance from the sterilisation facility to the release site, requiring about 12-13 h, and temperature fluctuations during the transportation, which could have facilitated some matings between the sterile insects (Nepgen et al., 2015). According to Nepgen et al. (2015), the cold temperature immobilisation and long-distance transport of irradiated *T. leucotreta* led to a decreased flight ability and longevity, although the realised fecundity was unaffected. Conversely, sterile *C. capitata* shipped as pupae from Mexico and Guatemala to South Africa, Argentina, Chile, Austria, Israel, and the continental USA, experienced no loss of adult quality, although the emergence was delayed by hypoxic conditions during shipping (Dowell et al., 2005; Rull et al., 2011). Similarly, Blomefield et al. (2011) reported that transporting codling moths, *C. pomonella*, as adults and pupae from Canada to South Africa for 67 h had little effect on moth emergence, longevity, and ability to mate, as assessed in the laboratory.

In summary, cold treatment is commonly used as a standard procedure to immobilise *T. leucotreta* before release (Boersma et al., 2019; de Villiers et al. 2019). This procedure facilitates the collection, handling, irradiation, transportation, and release of *T. leucotreta* (Boersma et al., 2019). However, chilling, long-term cold-temperature storage, and long-distance transportation may negatively impact moths' field performance by allowing

unwanted pre-mating before release, as shown in our study. Nevertheless, XSIT follows rigorous quality control and cold-transportation protocols aiding in the production of high-quality sterile *T. leucotreta*, although some mating incidences were still reported (XSIT, 2018). The main goal of the *T. leucotreta* SIT programme is to ensure a high percentage of mating between the released sterile and wild *T. leucotreta* populations to reduce reproduction below an economic threshold. However, our study found that sterile males and females mated before release and could potentially affect the technique's effectiveness by reducing the number of *T. leucotreta* acting as a positive sperm sink. Therefore, the results obtained from the study are valuable for XSIT to enhance the quality control of *T. leucotreta* by adjusting the temperatures at different production and release stages to minimise the sterile male and female matings, without affecting the overall fitness of the sterile *T. leucotreta*. However, no minimum threshold level of pre-release mating has been determined to help XSIT enhancing a better-quality control of sterile moths. Our findings support the continued improvement of the efficacy and effectiveness of the SIT programme as a control and management strategy for *T. leucotreta* in South Africa.

CHAPTER 7

GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

7.1 Introduction

Thaumatotibia leucotreta poses a major quarantine threat to South African fruit export markets such as the USA, EU, and the Far East (EFSA, 2024; Aigbedion-Atalor et al., 2024a). While it is endemic to SSA and African islands, there is a perception that the risk of it spreading to other regions of the world is increasing due to rising cross-border trade and global warming, which may allow it to survive in previously unsuitable areas (Adom et al., 2021; EFSA, 2024). Consequently, most export markets have implemented stringent proactive quarantine measures (Moore, 2021). This has led to losses in the South African citrus industry, both from direct fruit damage and indirect losses from consignment rejections when live specimens are detected (Moore, 2002; Hart, 2012; Mkiga et al., 2019).

However, more recent reports indicate significant improvements in controlling *T. leucotreta* due to the judicious use of various integrated management strategies (Moore & Hattingh, 2012; Barnes et al., 2015; Moore et al., 2015). When correctly managed and integrated, these control options have been shown to reduce *T. leucotreta* infestation by at least 97% (Moore et al., 2015). As a result, since 1960, the pest has transitioned from being an economic concern to a phytosanitary concern in the South African citrus industry (Hofmeyr et al., 2015; Moore, 2022). This has led to extensive research at CRI and other related organisations to mitigate this pest. Despite many attempts to control *T. leucotreta* using methods such as orchard sanitation, attract-and-kill techniques, mating disruption, biological control, and sterile insect techniques in citrus orchards, it remains a key phytosanitary pest in the industry (Hofmeyr et al., 2015; Huisamen et al., 2022; Moore, 2022; Githae et al., 2024).

SIT is an AW-IPM strategy used to control *T. leucotreta*. It is environmentally benign, species-specific, non-transgenic, and can be integrated with other management strategies (Vreysen et al., 2006; Mounika et al., 2022). Since insects do not recognise borders or farm boundaries, the implementation of an AW-IPM approach is essential. Modern integrated pest management focuses on holistic agroecosystem management, understanding the complete ecology of the target pest and its interactions with environmental factors (Conlong & Rutherford, 2009; Mounika et al., 2022). SIT involves mass-rearing, sterilising, and releasing large numbers of sterile males of the target insect into the wild to outcompete wild males and mate with wild females (Klassen, 2005). This results in a reduction of the wild fertile population, as the intrinsic rate of pest population increase is lowered. The main objective of a SIT programme is to induce sterility in the wild fertile *T. leucotreta* population as a control measure (Hendrichs et al., 2002). However, the success of the *T. leucotreta* SIT

programme depends on factors such as the fitness of sterile male *T. leucotreta*, their mating competitiveness and the overflooding ratio used.

The objectives of this study were: (i) to compare the efficacy and rate of population growth resulting from releases of sterile moths at a ratio higher than 10:1 (sterile: wild) with that achieved with the current benchmark of 10:1, in the laboratory and field cage studies, (ii) to compare fruit infestation in the laboratory and field cage studies, resulting from different combinations of treated (T) and untreated (U), male (M), and female (F) moths; UM×UF, TM×UF, UM×TF, TM×TF, and UM×UF×TM×TF, (iii) to investigate the acceptability and suitability of *T. leucotreta* eggs resulting from higher release ratios of sterile to fertile moths to parasitism by the egg parasitoid, *T. cryptophlebiae*, in a laboratory cage study, and (iv) to determine the pre-release mating levels across different stages of production and release of *T. leucotreta* in a SIT programme.

This chapter summarises and discusses the findings obtained from Chapters 2, 3, 4, 5, and 6 and provides recommendations for future studies towards developing an AW-IPM programme incorporating SIT against *T. leucotreta*.

7.2 The effect of overflooding ratios in a SIT programme

The success of a SIT programme depends greatly on factors such as fitness, mating compatibility, mating competitiveness, and the overflooding ratios of the released male *T. leucotreta* (Mounika et al., 2022). Since the *T. leucotreta* SIT programme relies on the mass release of sterile, mass-reared *T. leucotreta,* selecting the appropriate overflooding ratio is a critical step in the implementation of the technique (Mastrangelo et al., 2018). Considering the different ratios (sterile: fertile *T. leucotreta*) reported from various field studies, this study aimed to investigate the effects of different release ratios higher than 10:1 on levels of fruit damage and on the competitiveness of sterile male *T. leucotreta* which in turn affected the population growth rate of a fertile *T. leucotreta* population. The study demonstrated that increasing the number of sterile male *T. leucotreta* led to a reduction in the percentage of damaged fruit, the mean number of larval entries per treatment, and the emerging F1 adults. Moreover, the study revealed that any release ratio could suppress the *T. leucotreta* fertile populations by increasing the chances of fertile-to-sterile pairings over fertile-to-fertile pairings. These findings conform with those of Hofmeyr et al. (2015), who reported that from 2007 to 2010 after the technique's inception in South Africa in Citrusdal region, pest population reduced dramatically, with wild populations in sterile release areas dropping 3-, 8-

, and 10-fold compared to the non-release areas. Additionally, pre-harvest yield losses decreased by 50%, 80%, and 93%, while post-harvest export fruit rejections in SIT areas decreased by 13%, 25%, and 38%, respectively, compared to the non-SIT areas (Hofmeyr et al., 2015).

Here release ratios higher than the ratio of 10:1 significantly increased the efficacy of the technique. Although Hofmeyr et al. (2005) indicated that a release ratio of 10:1 was effective in reducing *T. leucotreta* fertile populations in citrus orchards, the laboratory study demonstrated that release ratios 40:1 and 60:1 can significantly reduce the proliferation of the fertile population more than lower ratios, leading to enhanced suppression of *T. leucotreta.* Additionally, field trials by Hofmeyr and Hofmeyr (2009; 2010) and Moore (2011) reported a release ratio of 40:1 in the citrus orchards, suggesting that it can improve the efficacy of the technique. Theoretically, a single fertile female *T. leucotreta* can lay 100-250 eggs, which can damage more than 100 fruit (Hofmeyr et al., 2015), and in Hofmeyr et al. (2005), 10 fertile female *T. leucotreta* were released per cage on each tree holding 50 fruit.

Notwithstanding the high damaging capability, up to 40% of fruit in the most promising treatment (150 Gy, 10:1 ratio) were protected from damage due to the release of sterile *T. leucotreta* (Hofmeyr et al., 2005). However, considering this high infestation capability of fertile *T. leucotreta*, this study demonstrated that 62% and 45% of fruit in this study were protected from infestation in release ratios 40:1 and 60:1. Similarly, Mastrangelo et al. (2018) found that sterility induction increased with the higher proportions of sterile *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae), with a 50:1 release ratio being effective in SIT field trials for the pest. Mudavanhu (2012) demonstrated a significant reduction in the mean number of damaged sugarcane stalks and F1 progeny in the treatments receiving sterile *Eldana sacharrina* (Walker) (Lepidoptera: Pyralidae) compared to the control cages. Flores et al. (2017) demonstrated that higher sterile-to-fertile ratios of the West Indian fruit fly, *Anastrepha obliqua* (McQuart) (Diptera: Tephritidae), lead to an increased sterility induction of around 80%, aiding in pest suppression. Cagnotti et al. (2016) demonstrated a significant reduction in the number of *T. absoluta* eggs and larvae as the overflooding ratios increased. Similarly, Jiang et al. (2023) recorded a notable reduction in both larval population and corn leaf damage as the release ratios of *S. frugiperda* increased, resulting in better pest control.

Despite the promising results from this study, increasing the ratio of sterile to fertile male *T. leucotreta* does not guarantee the success of the technique, especially if the released sterile insects are unfit and cannot outcompete the wild fertile males to mate with the wild

females (Boersma, 2021). The abundance of sterile *T. leucotreta* alone is insufficient; the mating compatibility between the sterile and wild fertile *T. leucotreta* is crucial for the technique's effectiveness (Shelly & McInnis, 2016). This necessitates supplementing the laboratory-reared population with fertile wild populations to prevent genetic inbreeding, which might lead to mating incompatibility between the populations. However, studies by Mgocheki and Addison (2016) indicated that *T. leucotreta* individuals could easily mate with those from different hosts and geographical areas, with mtDNA analysis showing they form a single group or clade. Similarly, Aigbedion-Atalor et al. (2024a) found that mating can occur among different geographically separated *T. leucotreta* populations. They observed that males tend to prefer females from their population when options are available. However, in situations where no choice is given, males successfully mated with and transferred spermatophores to females from all other populations. Furthermore, Aigbedion-Atalor et al. (2024b) showed that while local adaptation and other selection pressures in different environments play significant roles in sexual communication and selection in *T. leucotreta*, they do not necessarily hinder the recognition of sexual signals and attraction between geographically separated populations.

This study also demonstrated that the ratio 60:1 exhibited the lowest per generation rate of increase, indicating that the fertile population of *T. leucotreta* could be suppressed by a mean reproductive rate of 0.4× per generation, resulting in a decline of the fertile population. Hofmeyr et al. (2005) compared two release ratios (5:1 and 10:1) with the control and found that a 10:1 release ratio produced the lowest population growth rate. As a result, they recommended this ratio as the standard for the commercial SIT program in South Africa, which has been in use since 2007. However, this study proposes that a release ratio exceeding 40:1 can enhance the effectiveness of the SIT programme against *T. leucotreta*. Despite the promising laboratory results, maintaining a 10:1 release ratio in orchards is costly if other control measures are not effectively implemented. Therefore, producing and maintaining release ratios higher than 40:1 would incur even greater costs, making it imperative to use other control measures to reduce the number of sterile insects needed to suppress *T. leucotreta*. Additionally, the findings were obtained from a confined and controlled environment, which might have improved the insect's longevity, facilitating more fertile-to-fertile pairings. Therefore, ensuring that sterile insects can survive and remain sexually active long enough in the field is crucial. If their longevity declines, the frequency of releases needs to be assessed and increased to maintain sufficient overflooding ratios at all times (Dowell et al., 2021). The success of the sterile *T. leucotreta* release programme depends on ensuring

that the release of pre-determined numbers of sterile moths into the orchards could guarantee a minimum sterile to fertile male benchmark overflooding ratio, as assessed by trap catches (Boersma, 2021). The study revealed that using higher release ratios than a ratio of 10:1 can enhance the efficacy and effectiveness of the *T. leucotreta* SIT programme, particularly 40:1 and above.

7.3 The effect of sterile and fertile *T. leucotreta* in a SIT programme

The *T. leucotreta* SIT programme uses dual-sex releases because separating the moths by sex is impractical, given the large quantities required to supply the citrus orchards. However, my study (Chapter 3) reported that the dual-sex release (UM×UF×TM×TF) led to a higher percentage of fruit damage, an increase in the mean number of larval entries, and emerging F1 progeny compared to other treatments receiving sterile *T. leucotreta*. However, *T. leucotreta* suppression can be achieved from this combination over several generations due to the presence of sterile moths. Nevertheless, the high percentage of damaged fruit, larval entries and F1 adult's emergence could be attributed to the fertile *T. leucotreta* released which had better mating competitiveness compared to the sterile moths, as sterilisation and transportation often degrade the sexual performance of sterile insects (Ikegawa et al., 2021). Typically, the consistent and large-scale release of sterile insects reduces the population of wild/fertile pests by preventing normal mating between wild/fertile males and females, ultimately eradicating the wild/fertile population (Hendrichs et al., 2002). Due to the high infestation potential of a single fertile moth (~100 fruit) (Hofmeyr et al., 2015), the presence of 10 fertile female *T. leucotreta* in this treatment cage could have led to greater fruit damage, resulting in more larval entries and emerging F1 progeny. Conversely, this was also investigated by van Steenderen (2017) in laboratory settings, suggesting that dual-sex release can be more beneficial in a *T. leucotreta* SIT programme than males-only releases, as the sterile females can act as additional positive sperm sink for the wild/fertile males.

This study was conducted in a confined and controlled environment, which may have increased the likelihood of fertile-to-fertile pairings occurring. This is because, in field cage trials using the cactus moth, *C. cactorum,* sterile dual-sex releases were found to be significantly more effective than sterile male-only releases (Hight et al., 2005). Additionally, other SIT projects that released both sterile males and females and resulted in successfully eradicating the target pests, such as melon fly, *Z. cucurbitae* (Ohishi et al., 2018), and the sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Apionidae), on Kume Island, Okinawa, Japan (Ikegawa et al., 2021; Himuro & Ikegawa, 2024). Conversely, a large-scale

field trial by Rendón et al. (2004) found that SIT was more effective against *C. capitata* with sterile male-only releases compared to dual-sex releases. They suggested that sterile females reduced the chances for sterile males to mate with wild females, thus lowering the effectiveness of pest control. This indicates that sterile females can positively influence SIT by depriving fertile males of resources, such as time and sperm transfer, and reducing mating opportunities with fertile females (Whitten & Taylor, 1970; McInnis et al., 1986; Ikegawa et al., 2021). Additionally, the study conducted by Flores et al. (2017) showed that there were no notable differences in the percentage egg hatch between dual-sex releases and male-only releases in a SIT programme targeting *A. obliqua*.

Higher fecundity and fertility were recorded in the dual-sex release combination compared to the single-sex treatment involving sterile and fertile *T. leucotreta*. This suggests that the pest infestation levels would be high, as supported by our findings in the mean number of larval entries and emerging F1 progeny. This could be attributed to the increased fertile-to-fertile pairings in these cages, leading to more matings and resulting in the production of viable eggs that hatch and can infest the fruit. However, the phenomenon of inherited sterility, which is crucial in the *T. leucotreta* SIT programme, could not be elucidated in this scenario because the study was conducted only over one generation from P1 to F1.

From the study, single-sex release showed more suppression than a dual-sex release, as supported by the per generation rate of increase, with the treatment TM×UF showing the lowest rate of increase from the P1 to the F1 generation. According to Rendón et al. (2004) and Alphey and Bonsall (2018), releasing only male Medflies is generally more efficient than releasing both sexes. A large-scale study on sterile Medflies quantified this efficiency as being three- to five-fold more per male. From my findings, any treatment involving treated males or females had a lower per generation rate of increase compared to the control, even in the dual-sex release treatment. The study demonstrated that any combination of treated and untreated moths suppressed *T. leucotreta* proliferation by providing an opportunity for sterile-to-fertile pairings over fertile-to-fertile pairings. These results align with the findings of Hofmeyr et al. (2005), indicating that cages receiving treated *T. leucotreta* with 150 Gy at a release ratio of 10:1 (treated: untreated moths) had the lowest rate of per generation increase, suggesting that the fertile population would experience a slight decline from the P1 to F1 generation. Similarly, this aligns with the findings reported in Chapter 2, indicating a release ratio of 60:1, having the lowest per generation rate of increase from the P1 to F1 generation (see Results section “2.3.2 fecundity and fertility”). Although less damaged fruit, larval

entries and FI adult's emergence was recorded in single-sex releases, dual-sex releases have been practised in citrus-growing regions since the technique's inception in 2007 in South Africa (XSIT, 2018) due to the impracticability of moth sorting. However, the moths have to be kept inactive (4-6°C) to minimise any activity which might result in pre-release mating or damage of the body parts such as the wings, as discussed in Chapter 5.

7.4 The effects of overflooding ratios and sterile and fertile *T. leucotreta* under field conditions in a SIT programme

The success of any SIT programme depends on several key factors, including overflooding ratios and the mating competitiveness of the released sterile insects (Shelly & McInnis, 2016; Mounika et al., 2022). In this study (Chapter 4), field cage experiments were conducted to assess the impacts of different release ratios on fruit damage and population growth rates. However, the field results were limited, showing no significant difference between the control and all other release ratios except for the 20:1 release ratio. These findings contrast with those from Chapter 2, where an increase in sterile *T. leucotreta* resulted in reduction in fruit damage and larval entries. Similar outcomes were reported by previous studies, including Bloem et al. (1999) on *C. pomonella*, Hight et al. (2005) on *C. cactorum*, Hofmeyr et al. (2005) on *T. leucotreta*, Flores et al. (2014) on *A. ludens*, and Shelly & McInnis (2016) on *B. dorsalis*, *C. capitata* and *Z. cucurbitae.* The various combinations of sterile and fertile male and female *T. leucotreta* did not show any significant effect on fruit damage and larval entries. This contrast with the findings in Chapter 3, where the presence of sterile insects resulted in reduced fruit damage and larval entries, as also observed by Bloem et al. (1999) and Moore et al. (2021). The results from this field trial are inconclusive and further research is needed to validate the laboratory findings.

7.5 The role of sterile *T. leucotreta* eggs on parasitism in a SIT programme

Carpenter et al. (2004) and Carpenter (2013) identified the potential advantages of integrating sterile insects with other pest management strategies. Knipling's (1992) population models suggested that combining the inundative releases of biocontrol agents with sterile insects could produce additive or synergistic effects. Similarly, Carpenter et al. (2005) highlighted the possible synergistic effects between inherited sterility in sterile insects and the action of natural enemies. Hence, the effectiveness of population suppression of *T. leucotreta* through SIT alone could be enhanced by integrating it with natural enemies such as the egg parasitoid *T. cryptophlebiae*.

This study investigated whether *T. leucotreta* eggs, emerging from release ratios higher than 10:1 and of varying ages, are suitable for oviposition by the egg parasitoid *T. cryptophlebiae*. Results showed that *T. leucotreta* eggs emerging from higher release ratios (40:1 and 60:1) were suitable for oviposition by *T. cryptophlebiae*, though significantly less so compared to the control at egg age 48 h. Additionally, older egg age (72 h) had significantly fewer parasitised eggs across all release ratios. This may be because younger eggs (24 and 48 h) contain more vitellus, the primary protein source in the host egg for the developing embryo, compared to older eggs (72 h), resulting in a higher preference by *T. cryptophlebiae* for these younger eggs (Hagler et al., 1992). These findings demonstrate that while SIT and biocontrol agents operate differently, SIT can increase the number of *T. cryptophlebiae*, through parasitism of eggs laid by sterile-fertile parings, and the effectiveness of *T. cryptophlebiae* can enhance the technique's efficiency by reducing the wild/fertile population. Carpenter et al. (2004) reported significant differences in the number of parasitised *T. leucotreta* eggs when the host cross, particularly the female, was sterile or when the host egg age exceeded 24 h. This suggested that *T. cryptophlebiae* would successfully oviposit, develop, and emerge from *T. leucotreta* eggs laid by the different crosses of sterile and fertile *T. leucotreta* in citrus orchards (Carpenter et al., 2004). Similarly, Botto and Glaz (2010) indicated that sterile codling moth eggs were suitable for oviposition and development of the egg parasitoids *T. cacaeciae* and *T. nerudai*. In Chapter 2, Results section "fruit damage, larval entries, and F1 adults", my findings showed that release ratios exceeding 40:1 could result in the suppression of *T. leucotreta*. Additionally, since eggs from sterile insects support the development of *T. cryptophlebiae*, combining these two tactics could therefore enhance the overall effectiveness of the programme.

Similarly, Bloem et al. (1998) demonstrated that releasing a combination of sterile codling moths and egg parasitoids in field cages resulted in less fruit damage compared to using each technique alone. Likewise, Wong et al. (1992) showed that the simultaneous release of the opiine larval-pupal parasitoid *Diachasmimorpha tryoni* (Cameron) (Hymenoptera: Braconidae: Opiinae) and sterile Medflies significantly reduced the mean number of adult Medflies recovered per kilogram of fruit sampled, highlighting an effective tactic for suppressing a wild Medfly population. Additionally, a study by Cancino et al. (2023) indicated that concurrent releases of the parasitoid, *D. longicaudata* (Ashmead) (Hymenoptera: Braconidae), and sterile *A. ludens* in a field cage resulted in about 98% suppression of *A. ludens* wild population compared to release of sterile *A. ludens* only, supporting the use of both tactics in an AW-IPM context. Cagnoti et al. (2016) demonstrated

that the mirid egg predator *Tupiocoris cucurbitaceus* (Spinola) (Hemiptera: Miridae) consumed significantly more eggs from sterile females (about 20% more) than from untreated females, suggesting that these two tactics can be combined to suppress *T. absoluta.* Carpenter et al. (1997) suggested that greater suppression could be achieved by combining egg parasitoid releases with the F1 sterility technique. F1 sterility is theoretically more effective than full sterility in reducing *T. leucotreta* population growth, due to the superior fitness and competitiveness of the partially sterile F1 generation (Carpenter & Sheehan, 1996; Carpenter et al., 2005). The production of sterile F1 eggs can also provide more potential hosts for *T. cryptophlebiae.* Consequently, the number of *T. cryptophlebiae* should increase even if the parasitism rate remains the same (host-density independent), regardless of whether additional *T. cryptophlebiae* are released (Carpenter & Sheehan, 1996).

The findings by Moreira et al. (2009) revealed that the host eggs emerging from sterile insects might be unsuitable for the development of more than one parasitoid due to their low quality. My study identified whether the host eggs are suitable for superparasitism, indicating a significant decrease in the number of superparasitised *T. leucotreta* eggs in the control, as the host egg age increased. However, there was no significant difference in the number of superparasitised *T. leucotreta* eggs across the different release ratios for all the host egg ages. These results conform with those of Carpenter et al. (2004), which showed that the number of superparasitised eggs decreased as the host egg age increased and when the eggs were produced by sterile female *T. leucotreta*, as this decreases the host egg quality.

The quality and age of eggs emerging from sterile *T. leucotreta* can influence the emergence of *T. cryptophlebiae* (Carpenter et al., 2004). The study hypothesised that eggs from release ratios higher than 10:1, depending on their age, would be suitable for the development of high-quality *T. cryptophlebiae*, with the younger eggs being preferred. In support of my predicted hypothesis, the study found that *T. leucotreta* eggs from different release ratios and egg ages significantly affected the emergence of *T. cryptophlebiae.* More parasitoids emerged from younger eggs (24 and 48 h) than older eggs (72 h) across all release ratios. This suggests that while all *T. leucotreta* eggs from different release ratios are suitable for parasitoid development, egg age is a critical factor. This might be due to external cues, which might indicate that the internal quality of the eggs potentially influences the parasitoid's selection of the host eggs (Cagnotti et al., 2016; Suárez et al., 2019). The nutritional quality of host eggs is a crucial factor affecting the parasitoid's choice, as the higher nutritional value of the host eggs leads to improved fitness for the parasitoid, including better survival and greater fecundity (Eubanks & Denno, 2000). My findings align with those

of Pizzol et al. (2012) and Bari et al. (2016). In contrast, Carpenter et al. (2004) reported a significant decrease in the number of *T. cryptophlebiae* emerging from the eggs laid by sterile *T. leucotreta* at egg age 24 h, possibly due to the low vitelline content of the eggs, hence low preference by *T. cryptophlebiae*. Additionally, the study found that host egg age affected the sex ratio of emerging *T. cryptophlebiae*, with more females emerging than males from young egg ages (24 h). This aligns with El Sharkawy (2011), who indicated that producing more female parasitoids is crucial, as they can continue laying eggs on available host eggs, resulting in population build-up, and can be easily fertilised by the few male individuals present. Similarly, Cancino et al. (2002) and Suárez et al. (2019) showed that the size and age of the host larva (*C. capitata*) influenced the decision of *D. longicaudata* females to lay male or female eggs, with smaller and older host instars typically resulting in a higher proportion of male offspring, in the mass-rearing of *D. longicaudata*.

The quality of the emerging parasitoid is crucial for maintaining the effectiveness of *T. cryptophlebiae* in controlling *T. leucotreta*. A flight test study was conducted to determine whether the emerging *T. cryptophlebiae* from host eggs of different ages, produced by sterile *T. leucotreta,* were fit and capable of flying to search for host eggs to parasitise. The proportion of flying *T. cryptophlebiae* was significantly influenced by the different release ratios and egg ages. The results indicated that egg ages 24 and 48 h produced a significantly higher proportion of flying parasitoids than egg age 72 h, across all ratios. Additionally, more female than male *T. cryptophlebiae* were capable of flight. Pérez-Staples et al. (2021) reported similar findings, demonstrating that flying parasitoids can easily disperse to find mates and host eggs to parasitise, thus aiding in parasitising more *T. leucotreta* eggs, lowering the pest population. The study also revealed that a significantly higher proportion of walking *T. cryptophlebiae* emerged from older eggs (72 h) compared to 24 and 48 h egg ages, especially at release ratios of 40:1 and 60:1 compared to 20:1, 10:1 and the control. This indicates that unfit, walking *T. cryptophlebiae* emerged from old, lower-quality *T. leucotreta* eggs. According to Soares et al. (2012), high flight capability is more beneficial than walking due to their greater mobility and higher likelihood of locating host eggs, aiding in parasitoid build-up. The study's findings support the population control model, showing that population suppression is enhanced when sterile insects (*T. leucotreta*) and parasitoids (*T. cryptophlebiae*) are released together, and that the percentage reduction in population growth is greater when parasitised hosts produce more flying parasitoids compared to when no parasitoids are produced (Carpenter, 2000).

7.6 The role of pre-release mating in a SIT programme

The *T. leucotreta* SIT programme involves releasing both sterile males and females into citrus orchards to control the wild population. Consequently, pre-release mating might occur, as sterile females may mate with sterile males, acting as a negative sperm sink (Neville Boersma, per. Comm.; van Steenderen, 2017). The study (Chapter 6) hypothesised that pre-release mating happens throughout the different stages of production and release, despite XSIT's efforts to control it. The ideal method to determine mating competitiveness is measuring spermatophore transfer to the female *T. leucotreta* bursa copulatrix (Moore et al., 2021). Using this method, the study's results supported the hypothesis, showing that in January, the mean number of spermatophores and mated females were significantly influenced by the different production and release stages. A significantly higher number of spermatophores and a percentage of mated females were recorded post-irradiation stage compared to the release stage in the Olifants River Valley (ORV) region, which had the lowest number of spermatophores transferred in January. Azrag et al. (2021) indicated that female *T. leucotreta* are polyandrous and start mating a few hours after eclosion. Similarly, significantly more spermatophores were reported in Sundays River Valley (SRV) compared to the ORV region. Although *T. leucotreta* released in the SRV region are transported by road for 12 h, the results confirm the findings by Nepgen et al. (2015) that while handling, cold immobilisation, and transport significantly affect the flight ability and longevity of sterile *T. leucotreta*, fecundity remains unaffected, and they remain active to mate.

The results indicated that in March, the mean number of spermatophores transferred and percentages of mated females were not significantly affected by different stages of production and release regions. This might be due to XSIT maintaining recommended conditions across all stages, resulting in low mating levels (XSIT, 2018). This could have aided in better field performance, as the sterile males conserved energy and time by not mating with sterile females (Boersma, 2021). However, the mean number of spermatophores and percentage of mated female *T. leucotreta* were significantly influenced by different production and release stages. In May, a significantly higher number of spermatophores and a greater percentage of mated female *T. leucotreta* were recorded in moths sent to the SRV region and post-irradiation. Conversely, the lowest mean number of spermatophores and mated female *T. leucotreta* were recorded during the eclosion stage. The overall findings from the study demonstrated that the mean number of spermatophores transferred and the percentage of mated female *T. leucotreta* were significantly higher post-irradiation and at the release stage in the SRV region. The study concluded that temperature fluctuations in the

production facility and during transportation can negatively impact the sterile *T. leucotreta*, resulting in mating among the sterile moths (Nepgen et al., 2015; Boersma & Carpenter, 2016; Boersma, 2021). These results reported here are important as they identify the stages at which high levels of pre-mating occur and can aid XSIT in monitoring and improving the quality of the sterile *T. leucotreta* released into citrus orchards. Nevertheless, de Villiers et al. (2019) demonstrated that mating before irradiation can increase female's fertility by 10 times. Although no concrete evidence is available from this preliminary study, if this was to happen in the field, a higher fruit infestation might be witnessed. However, Bakker et al. (2011) suggested that multiple matings in insects like *T. leucotreta* can negatively impact their fitness, as well as reduce their mating vigour and this can affect the success of the technique.

Given the impracticality of sorting of moths due to the large numbers required in the field, dual-sex releases will remain the standard practice. Consequently, some level of pre-release mating is unavoidable, making it crucial to establish the tolerance threshold levels for XSIT to maintain the technique's effectiveness. Since this study has identified the stages with higher pre-release mating, further research is needed to determine the underlying causes.

7.7 Recommendations

The study detailed in Chapter 2 explored the effects of release ratios higher than 10:1 on fruit damage and population growth. However, the study was limited to a controlled environment (CE) room. Field trials were conducted; however, they proved unsuccessful, yielding limited results. Therefore, more field trials need to be conducted to validate the laboratory results. A cage trial by Hofmeyr et al. (2005) found that a 10:1 ratio is effective for controlling *T. leucotreta* and was set as the benchmark. However, the cost of mass-rearing, sterilising, and transporting *T. leucotreta* at this ratio is expensive. This study determined that ratios 40:1 and 60:1 can also be effective for *T. leucotreta* suppression. Thus, the study recommends that successful implementation of other control measures to further achieve *T. leucotreta* suppression while still maintaining these high release ratios. SIT is not a standalone technique and is dependent on integration with other suppression methods for successful control of *T. leucotreta* (Nepgen et al., 2018). Therefore, it would be wise to investigate whether the integration of higher release ratios 40:1 and 60:1 with selective insecticides or mating disruption can improve the suppression of *T. leucotreta*.

Chapter 3 of this thesis explored the effects of different combinations of sterile and fertile *T. leucotreta* (Meyrick) on fruit infestation and population growth rate. The study was conducted in a CE room under optimal conditions. Field trials were also conducted; however,

they proved unsuccessful, yielding limited results. Therefore, field trials need to be conducted to validate these laboratory results. Additionally, according to Bloem et al. (2003), any pairing between a sterile male and female *T. leucotreta* should produce non-viable eggs, as sterile females are completely sterile. However, my study recorded some fruit infestation in these pairings. Therefore, the study recommends more trials on this be conducted to ensure that the radiation dose used by XSIT to sufficiently introduce complete sterility in females. Moreover, the use of maternally transmissible *Wolbachia* bacteria as a biological intervention for pest suppression has been explored in pests like the African armyworm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae) and Medfly (Ahmed et al., 2015). Combining SIT with *Wolbachia* symbiosis has been proposed as a potential control method for various pests such as Medfly and *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) (Nikolouli et al., 2018). Wolbachia has been found in at least 300 different lepidopteran species (Ahmed et al., 2015). While *Wolbachia* compatibility has not been tested in *T. leucotreta*, the infection incidence is likely to be high (Ahmed et al., 2015) and, therefore, may play a significant role in future IPM programmes, improving their efficacy.

Chapter 4 of this thesis presented limited findings from the field cage experiments. Therefore, the study recommends selecting of a citrus variety that is strongly preferred by the *T. leucotreta* and replication of the study across several citrus orchards. It also suggests conducting two or more releases rather than a single release to increase the number of sterile insects released per cage while maintaining the initial release ratios. Ensuring the release of high-quality moths is crucial, as this might affect the moth performance in the cages by lowering their mating competitiveness. Furthermore, microclimatic conditions within the cages needs to be investigated, as they could have some confounding effects on the moths.

Chapter 5 of this thesis explored the potential for integrating higher release ratios of sterile to fertile *T. leucotreta* and the egg parasitoid *T. cryptophlebiae*. The findings suggested that *T. cryptophlebiae* could successfully oviposit, develop, and emerge from *T. leucotreta* eggs laid by various crosses under a sterile insect release programme for *T. leucotreta* (Carpenter et al., 2004). However, these experiments were conducted in a CE room under optimal conditions. Therefore, these results suggest that additional field cage and open-field evaluations are necessary to test the efficacy of combining sterile *T. leucotreta* and *T. cryptophlebiae* releases. Additionally, flight activity is a crucial trait for assessing the performance of parasitoids in field conditions and serves as a good indicator of their quality (Prezotti et al., 2002). However, additional biological variables need to be monitored to ensure the production of high-quality *T. cryptophlebiae*. Consequently, the study

recommends following IOBC guidelines. Other biological parameters such as longevity, parasitism index and adult size of *T. cryptophlebiae* also need to be investigated.

Improvement and standardisation of quality control protocols is a necessary step towards improved SIT and should be implemented for both the product (the target insect) and the overall process used to rear, sterilise and release the sterile insect (Robinson & Hendrichs, 2005). Proper temperature management from moth collection to release is of vital importance for sterile insect performance in the field, and failure to maintain the prescribed cold chain can result in unacceptable deterioration of both moth quality and competitiveness (Boersma & Carpenter, 2016). Therefore, more emphasis should be put on this to ensure the moths are inactive but still maintain their quality. Chapter 6 findings conducted from January to May, are essential for XSIT to identify areas for improvement in the production and transportation of sterile *T. leucotreta.* Therefore, the study recommends conducting more pre-mating release trials throughout the entire citrus production season to determine how pre-mating levels vary over time. Since *T. leucotreta* releases involves both sexes, pre-release matings is inevitable. Thus, more research is required to establish the minimum acceptable pre-mating thresholds that can be tolerated by XSIT while still preserving the high quality of the moths.

7.8 Conclusions

The thesis presented some insights into the effects of overflooding ratios, combinations of different sterile and fertile *T. leucotreta*, integration of *T. cryptophlebiae* with SIT, and the role of pre-release matings in a *T. leucotreta* SIT programme. Key findings suggest that release ratios exceeding 40:1, and combinations of sterile and fertile male and female *T. leucotreta*, particularly the TM×UF combination, could effectively suppress *T. leucotreta* in citrus orchards. However, any release ratio and combination of sterile and fertile *T. leucotreta* can still exhibit some suppressive effects due to sterile-to-sterile matings. Furthermore, integrating SIT with *T. cryptophlebiae* could further improve the effectiveness of the *T. leucotreta* SIT programme, as *T. leucotreta* eggs from different sterile crosses could support *T. cryptophlebiae* development, indicating the compatibility of these two tactics in suppressing *T. leucotreta*. The mating competitiveness of sterile moths in dual-sex releases can be affected by pre-release matings, necessitating increased monitoring and precautions to improve moth quality. The findings should be further studied and incorporated into commercial SIT programmes for better *T. leucotreta* control. Additionally, the results could inform other SIT programmes, aiding in enhancing the activity and competitiveness of various insect species.

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